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Arthur C. Walton

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SOME PARASITES AND THEIR CHROMOSOMES*

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Soon after the invention of the microscope, curiosity led investigators to hunt for smaller and smaller units of living matter; whether these were entire organisms or portions of larger forms seemed to have made little difference. Out of that search arose the first knowledge of the protozoa, of bacteria, and much later, of still smaller organisms. The concept of the existence of cells as a unit of structure led eventually to the announcement of the Cell Theory, applying equally to both plant and animal organisms, about 120 years ago. Some organisms were regarded as single cells and others as being composed of groups of cells, each showing various degrees of independence or of interdependence. This step was followed by the recognition of certain definite structures as being characteristic and integral parts of the living cell.

From such a beginning came the study of cellular morphology and the recognition of such cellular regions as the nucleus and its contained chromatic materials, often in the form of organized bodies called chromosomes. With the appreciation of the fact that the chromosomes behaved in a more-or-less predictable manner during the life of the cell, details of mitosis and later of meiosis were worked out, and with the determination of the potential individuality of the chromosomes arose the concept of certain of them being connected, not only with the general matter of inherited characteristics, but also with sexual differentiation.

Geneticists have made use of these bodies in their studies and the whole picture of gene location has developed. Not only the physical structure but also the chemical nature of the chromatin bodies, indeed of the whole nuclear apparatus, has become a fertile field of research for many present-day students of cellular organization.

With the realization that the chromosome number of any particular organism is constant within certain definite limitations, came the belief that it might be possible to predict the sex of the adult form by studying the chromosomes of the embryo, and in cases where there was a sexual differentiation in chromosome number, pattern of distribution, or behavior, it would be possible to determine what combination of sex cells would result in an embryo of a particular sex.

My own interest in cytology began at a time when the concept of the so-called "X" chromosome was just becoming established through the work of McClung and his students, and encouraged by the advice of Boveri and Marcus to work on *Ascaris* material, my investigations on nematodes and their germ cycles were initiated. Work under Mark at Harvard on the embryology of certain nematode species led to the realization that forms other than *Parascaris equorum* underwent that peculiar phenomenon known as "diminution," or the loss of chromatic material (whole chromosomes?) during early embryonic development. Subsequent investigations

* Address of the retiring President, Bloomington, Indiana, August 27, 1958.

by many researchers has shown that much material, both particulate and in solution, is eliminated from the nucleus at various times, and frequently such substances are chromatic in origin.

Further efforts to ascertain the presence or absence of this phenomenon of "diminution" and of the presence or absence of "sex chromosomes" led to the examination of many species of nematodes and to the determination of their chromosome number and behavior, especially during gametogenesis. The maintenance of some particular number of chromosomes among related species or even genera of nematodes, and also certain similarities in the form, size, shape and/or behavior of some of these same chromosomes caused speculation as to whether such consistencies might not be of taxonomic value. Other workers in the field, some dealing with trematodes, some with cestodes, and some with protozoans, have ventured similar suggestions from time to time. The literature records similar observations based on studies of almost every group, plant or animal, free-living or parasitic. Such a suggestion of taxonomic utilization of chromosomes to indicate possible evolutionary relationships is, to my mind, still tentative. There seem to be so many more exceptions than there are conformities to such a pattern that any sweeping generalizaions would be of very doubtful validity.

Examination of over 50 species of nematodes, together with later observations by other workers gives available data on 1 genus and 1 species of the Desmadoridae, 1 genus and 4 species of the Rhabditidae, 1 genus and 4 species of the Rhabdiasidae, 1 genus and 2 species of the Strongyloididae, 5 genera and 11 species or subspecies of the Ascaridae, 1 genus and 3 species of the Anisakidae, 1 genus and 1 species of the Kathlaniidae, 2 genera and 2 species of the Oxyuridae, 2 genera and 6 species of the Heterakidae, 3 genera and 5 species of the Strongylidae, 1 genus and 1 species of the Heligmosomidae, 2 genera and 2 species of the Trichostrongylidae, 3 genera and 4 species of the Metastrongylidae, 1 genus and 1 species of the Camallanidae, 1 genus and 1 species of the Rhabdochonidae, 1 genus and 1 species of the Acuariidae, 2 genera and 2 species of the Spiruridae, 2 genera and 2 species of the Physalopteridae, 1 genus and 1 species of the Setariidae, and 1 genus and 1 species of the Trichosomoididae.

Over one-half of these forms show a somatic number of chromosomes greater than the number present in the zygote or in the "stem" cells of the embryo, and supports the postulation that the germ-cell-number is composed of compound chromosomes in many instances. Some authors, however, interpret this phenomenon as indicative of fragmentation during the formation of the somatic chromosomes (in *Parascaris equorum* the diploid number may be *2* or *4* or *6* depending upon the variety being studied, and the somatic number of chromosomes may amount to rather high figures). "Diminution," or the loss of chromatic material is known to occur in at least 9 species of nematodes, as well as occurring in other phyla such as the Protozoa, the Arthropoda, and the Chordata. This loss has been interpreted as an attempt to stabilize the embryonic environment of the "stem" or "germ-track" cells during development (Ubisch, 1943). It is known that DNA is released from the nucleus at this time and such more-or-less universal release of nuclear chemicals may on occasion have a physical manifestation (particulates). Such a release may also be associated in some manner with the so-called "chromosome fragmentation" noted in some somatic cells. Lin, 1954, suggests a comparison of this elimination of chromatic material (heterochromatin is mainly DNA) from the collective germ-cell chromosomes to the macronuclear phenomena noted in so many protozoa. Ex-

amination for the presence or absence of recognizable "sex chromosomes" (heterochromosomes) has afforded records indicating that 37 species of nematodes rather definitely show the presence of an "X" chromosome—sometimes appearing as a complex of 2 or more members in certain species—and in 6 species the presence of a "Y" chromosome (however in only one species, *Contracaecum incurvum,* has subsequent work substantiated the presence of such a "Y" element). Jeffrey and Haertl's 1938 objection to interpreting apparently unpaired and lagging chromosomes as being heterochromosomes has not held up in the light of more recent investigations (Nigon and Robert, 1952, and Lin, 1954). Heterochromosomes may appear in forms in which the sperm acts only as a stimulating agent (true fertilization never taking place) and also in parthenogenetic species (Nigon and Roman, 1952, in *Strongyloides ratti*).

From the taxonomic standpoint, the number and behavior of the chromatic elements seem to have a great deal of uniformity within species of a single genus, and even among species of closely related genera, and therefore may be of some significance. In the first place the so-called "diminution" phenomenon is found only in members of the Ascaridata (Nematoda), primarily among the Ascaridae. Secondly, the number of the so-called "X"-bodies is greater than *1* only in the Ascaridata ("X" may be composed of *1, 2, 5, 6,* or *8* units). The presence of an undoubted "Y"-body is reported only from the Ascaridata (*Contracaecum incurvum* by Goodrich, 1916). Thirdly, there is a certain consistency of total chromosome numbers in various groups. The Strongyloididae have the reduced number of *3;* the Rhabdiasidae all show the haploid number of *6;* the Rhabditidae as a rule have $n = 7$, although one form has $n = 9$; 11 of the 12 species of the Strongylata show $n = 6$ (one has $n = 8$); the Heterakidae seem to group around $n = 5$, and the Oxyuridae around $n = 4$ (occasionally *8*). Most of these groups are quite homogeneous and the similarity of chromosome numbers could be expected; on the other hand, the Ascaridata are much more heterogeneous in composition and the wide variation in chromosome numbers should equally be expected. These variations as found within a closely knit group have been interpreted as examples of fragmentation or duplication of one or more chromosomes (aneuploidy) rather than as cases of duplication of sets of chromosomes (polyploidy).

The closely related Nematomorpha show a somewhat similar situation in that members of the genus *Gordius* show $n = 2$ or $n = 4$. *Paragordius,* however, has $n = 7$; a situation not in keeping with the interpretation of close relationships being indicated by the number of chromosomes present.

When one turns to the Platyhelminthes, one finds a somewhat similar picture, although this phylum is far less homogeneous than the Nematoda, with such variant groups as the non-parasitic Turbellaria and the parasitic Trematoda and Cestoda.

Among the turbellarians, records of chromosome numbers among the primitive Acoela are few, but 3 species of the Convolutidae have been studied and all seem to indicate that the somatic number of very small chromosomes is somewhat variable but high—ranging from *20* to *30*.

Records for the other orders of this basically hermaphroditic and occasionally parthenogenetic class are numerous and the results have been corroborated by various researchers.

Among the Alloeocoela, members of the Prorhynchidae, the Plagiostomidae, and the Bothrioplanidae usually show a basic haploid number of *10* (reduced to *5* in one

species), but the Monocelididae have the haploid number of *3*, while the Pseudostomidae have either *4–5* or *8–10*. Since polyploidy is common among the turbellarians, the basic haploid chromosome number for the alloeocoels may be *5*, although *Monocelis fusca* ($n = 3$) does not readily fit into the schema.

Among the Tricladida there seems to be a much greater degree of variation in the basic haploid number of chromosomes. Among the Planariidae the number ranges from *6* to *24* (*6, 8, 12, 16, 18,* and *24* have been recorded). *Curtisia* and some polycelids have $n = 6$. In other polycelids polyploidy is believed to give rise to the species having $n = 12, 18,$ or *24*. Lepori (1953a, b) believes that in some cases among the polycelids true fertilization does not occur, and that the multiples of the basic chromosome number may perhaps be due to failure to undergo reduction divisions (a condition Lepori terms gynogenesis). In the genus *Planaria* most of the species have the basic chromosome number of *8*. In the genus *Dugesia*, the number varies from *8* to *16* or to *24*, again an example of possible polyploidy, but the appearance of *12* chromosomes in *D. alpina* may be an indication that the basic number is *4* rather than *8*, as stated by Benazzi-Lentati, 1949.

Among the Dendrocoelidae we again find considerable variation in the basic haploid number. *Dendrocoelum* has species with *8* and others with *16* chromosomes (possible examples of polyploidy?). *Procotyla* and one variety of *Dendrocoelum lacteum* (both with *7* haploid chromosomes) and *Bdellocephala* with *10* chromosomes are variants (possible aneuploidy?). Other families such as the marine Bdellouridae and the Procerodidae are represented by too few species in which the basic chromosome number has been determined (*Bdelloura*, where $n = 6$ and fragmentation may raise the number as high as 12; and *Procerodes*, with $n = 6$) to be indicative of their evolutionary position, although the presence of fragmentation may point toward a possible compound nature of the gametic chromosomes.

Turning to the Polycladida we again note a variability in the haploid chromosome number which ranges from *6* to *8* and occasionally to *9* or *10*. The Euryleptidae, the Leptoplanidae, and the Prosthiostomidae seem to have the basic number of *8* while the Planoceridae possess *10* chromosomes, but no such consistency appears among the genera of the Stylochidae where the basic numbers are either *9* or *10*. The single pseudocerid examined has *9* chromosomes. It might be suggested that in these cases possible fusion of some of the chromosomes may have occurred, thus reducing the number from *10* to *9*, or even to *8*, and thus showing relationship to the basic *10* pattern set by the Planoceridae as a whole. An alternative possibility is that a doubling of one or more chromosomes has caused the deviation from a basic number of *8*, as represented by the Euryleptidae. On purely morphological grounds the planocerids are regarded as the more primitive and the euryleptids as more specialized among the polyclads.

Among the higher Rhabdocoela, we again find considerable variation in numbers, but the basic count is low, ranging from *2* to *6*, and it is suggested that fragmentation of one or more chromosomes plus some group duplication of the basic number may be involved. Among the supposedly more primitive rhabdocoels (Catenulidae) the basic number runs much higher, ranging from *15* to *20* and the somatic numbers may reach to *40* [*Stenostomum, Rhynchoscolex, Catenula* and *Fuhrmannia* = *Suomina* are representative genera]. According to Pennypacker, 1954, such variation is of evolutionary importance in that as a general rule a larger number of smaller chromosomes is indicative of ancestral conditions and a smaller number points toward a more recently emerged species. Among the Typhloplanidae

it may be noted that *Opistomum, Krumbachia, Solenopharynx, Amphibolella,* some protoplanellids, and *Phaenocora,* as well as the North American form of *Rhynchomesostoma rostratum* show a basic number of *2*. (The European race of *R. rostratum* has *3* chromosomes, as do *Trigonostomum,* some protoplanellids, and *Costrata.*) Papi, 1950, indicates that the basic number for the Mesostominae is *4*, but through fusion, fragmentation, or ploidy, some species show $n = 2, 3, 5,$ or *8*. The Dalyelliidae all show $n = 2$. Jones, 1944, has indicated that among the Macrostomidae, the basic number is probably *3* and that a few species show multiples in the form of *6* and *9* as the reduced number of chromosomes (some species however, show *2* and *8*). The Graffillidae also show—at least in the genus *Paravortex*—that the basic number is *2*, doubled in one species to *4*. The Provorticidae have a basic number of *3*. The Kalyptorhynchidae vary from *2* to *3* to *8*. The single temnocephalid examined has *8* chromosomes. As yet there seems to be no complete agreement between chromosome number and the phylogenetic position of these forms as determined by other criteria, but on the other hand no absolute contradictions have come to light.

Although the turbellarians are not parasitic except for a few possible exceptions, they have been introduced into this discussion because they do show the gradual reduction of the basic number of chromosomes from a large number of small chromosomes to a smaller number of larger units, not only within the group as a whole, but in some cases within races of the same species, and may possibly give hints as to the derivation of the Trematoda which do show a much greater conformity between the chromosome number and the taxonomic position as based on other criteria. There seems to be considerable evidence already available pointing to the trematodes as evolving from a rhabdocoel stock, and possibly from the dalyelliid group. Cytological evidence seems to corroborate this interpretation of a rhabdocoel ancestry, but if such development came through the dalyelliids, it must have been from a stock much less specialized than the present-day forms which are regarded by many as being among the more advanced of the rhabdocoels. In this same connection it may be of interest to point out that the acoelids and the alloeocelids are regarded as the most primitive of the turbellarian line of development and most consistently possess the highest basic number of chromosomes (*10* in most of the species examined). Through some form such as *Bothrioplana* ($n = 10$) could have come the triclads (some of the dendrocoelids show this basic number of *10*). Polyclads and the rhabdocoels lack such close cytological connection with a possible alloeocoelid ancestry although some of the polyclad planocerids and some of the rhabdocoelid macrostomids do show practically the same basic chromosome numbers.

Since both the Monogenea and the Digenea among the trematodes are believed to have been derived independently from a rhabdocoel stock, examination of their chromosome numbers may be of some value. A number of workers on trematode cytology, especially Jones and his co-workers, have pointed out the possible taxonomic value of the study of the numbers, volume, and/or size and shape of chromosomes, and much of our information along these lines is based upon their investigations.

Among the Monogenea only the Polystomidae have been investigated for the purpose of determining the chromosome numbers. *Polystoma integerrimum* has the haploid number of *4* chromosomes and *Gyrodactylus elegans* has *6*. This could point to a dalyelliid ancestry ($n = 2$ in all species studied) although more primitive ancestors of the dalyelliids may have had a larger basic number of chromosomes.

Among the Digenea, the paramphistomatids have been regarded as the most primitive group, but from a cytological standpoint the basic chromosome number is quite variable and is therefore of little guidance in determining possible relationships. For example, *Gigantocotyle* shows a basic number of *6*, *Gastrothylax* and *Zygocotyle* have *7*, *Cotylophoron* and *Diplodiscus* have *8*, while *Heronimus* (*chelydrae*) has *10*. No one family of the rhabdocoel group could be regarded as being directly ancestral, based on such divergent records.

Inasmuch as the exact phylogenetic relationships of the remaining families of the Digenea are not as yet fully determined, even on morphological grounds, and the various authorities disagree as to their proper taxonomic positions, no attempt will be made to discuss our knowledge of chromosome numbers in any significant succession. It may be noted, however, that all of the heterophyids (*Cryptocotyle* and *Acetodextra*), bucephalids (*Bucephalus* and *Rhipidocotyle*), fasciolids (*Fasciola*), and zoogonids (*Zoogonus*) examined (7 species), as well as 1 species of a gorgoderid (*Probolitrema*) and 1 of a paramphistomatid (*Gigantocotyle*) have a basic number of *6* [perhaps thus indicating some relationship to the more primitive paramphistomatids ($n = 7$)]. The notocotylids (*Notocotylus*) have *7* chromosomes, but too few examples have been studied to determine the value of such counts. One species of an allocreadid (*Bunodera*), 1 gorgoderid (*Gorgoderina*), and 1 schistosomatid (*Schistosomatium*) have *7* chromosomes, in addition to the 2 amphistomids (*Zygocotyle* and *Gastrothylax*). All of the *Schistosoma* species studied show $n = 8$, as do 2 species of gorgoderids (*Gorgodera* and *Phyllodistomum*), 1 troglotrematid (*Paragonimus*), 3 species of allocreadids (*Bunodera, Crepidostomum* and *Allocreadium*), 1 species of a rhopaliid (*Rhopalias*), and 2 species of the reniferids (*Staphylodora* and *Telorchis*), in addition to the 2 species of paramphistomatids (*Cotylophoron* and *Diplodiscus*). Two species of the azygiids (*Azygia* and *Proterometra*), 2 species of the plagiorchids (*Eustomas* and *Glypthelmins*), 1 species of a spirorchid (*Spirorchis*), 1 pronocephalid (*Macrovestibulum*), 1 lecithodendriid (*Brandesia*), 1 monorchid (*Asymphylodora*), 1 hemiurid (*Halepegus*), and 1 renniferid (*Auridistomum*), in addition to 1 race of the paramphistomatid *Diplodiscus* (*temperatus*) have *9* chromosomes. Those having a basic number of *10* chromosomes are 1 species of a cyclocoelid (*Cyclocoelum*), 2 species of dicrocoelids (*Brachycoelium* and *Dicrocoelium*), 1 clinostomatid (*Clinostomum*), and 1 hemiurid (*Isoparorchis*). Only *Heronimus* of the paramphistomatids falls in this category. Four species of plagiorchiids (3 *Pneumonoeces* and 1 *Plagitura*), 1 echinostomid (*Parorchis*), 2 lecithodendriids (*Acanthatrium* and *Loxogenes*), and 15 species of reniferids (1 *Dasymetra*, 1 *Lechriorchis*, 1 *Natriodora*, 6 *Neorenifer*, 2 *Pneumatophilus*, 1 *Renifer*, and 3 *Telorchis*) have *11* chromosomes. In these cases again no direct relationship to the paramphistomatids can be noted in terms of chromosome number, although in terms of the presence of an increased number of chromosomes as indicating a possible primitive condition, these forms might be regarded as less specialized than others of the Digenea. It should be pointed out, however, that in many cases it is not only in the matter of actual number of chromosomes that similarities (relationships ?) may be indicated: total volume of chromatic material, shapes and sizes of the chromosomes, point of spindle attachment to individual chromosomes, and behavior during division may afford evidence of equal importance. It is also definite that sufficient differentiation occurs to facilitate identification of species. One species of *Cephalogonimus* has *14* chromosomes which

may be a case of doubling of the usual number of *7* in this genus. If one follows the belief of Ciordia (1949), who doubts the existence of polyploidy among the Trematodes, this would be an example of extreme aneuploidy—duplication of individual chromosomes and not of the set as a unit.

The matter of the presence or absence of the so-called heterochromosome ("sex chromosome") has not been determined in most of the trematode species examined, but in a few cases the recognition of two types of sex cells differing from each other in terms of the number, size, shape, volume or behavior of the chromosomal elements would seem to indicate that such sexual differentiation does occur. As examples we may mention the studies on *Schistosoma* and *Schistosomatium*. The earlier observations on *Schistosoma haematobium, S. mansoni,* and *S. japonicum* seemed to indicate that two types of sperm could be identified and that adult males possessed *15* and adult females possessed *16* somatic chromosomes. This would seem to mean that an *X–0* condition obtained in these forms. Other studies reported the numbers as *14* and *16,* respectively, and were interpreted as showing the presence of a $2X + 12$ and a $4X + 12$ chromosome complex. Niyamasena (1940) in his studies on *S. mansoni* found the somatic number of chromosomes to be *16* in each sex, and could not find any evidence of the presence of recognizable sex chromosomes although the possibility of an *X–Y* condition could not be ruled out. Most recent studies support the finding of *16* chromosomes as the diploid number in all adults of all three of the above *Schistosoma* species and the inability to recognize sex chromosomes as being present [16 chromosomes are also present in each sex of *S. mansoni* carcariae (Short and Menzel, 1957)]. Recent studies on *Schistosomatium douthitti* have presented 2 different interpretations of what is undoubtedly an example of the presence of distinguishable sex chromosomes. One study (Woodhead, 1957) indicates the presence of a single "X" chromosome in the male, while the other (Short, 1957) presents evidence of the heterogametic condition as prevailing in the female. Studies in this laboratory seem to substantiate this second interpretation. The somatic (diploid) number of chromosomes in each sex is *14,* with the male showing a pair of large V-shaped chromosomes that are not matched in the female. In the latter case there is a single large V-shaped chromosome apparently paired with a single rod-shaped body. This rod-shaped chromosome does not appear in any of the male cells. It is interpreted as indicative of a *ZZAA* condition in the male and a *ZWAA* condition in the female. Short and Menzel (1957) report a similar condition in the cercariae of *Ornithobilharzia canaliculata,* where $2n = 16$. No other records of the presence of recognizable sex chromosomes among the trematodes seem to have been substantiated by recent investigations. No records of the "diminution" phenomenon have been brought to our attention.

Le Roux (1958) in a study of mammalian blood flukes, has suggested a division of the genus *Schistosoma* into several groups: *Schistosoma* (*S. haematobium,* type), *Afrobilharzia* (*A. mansoni,* type), *Sinobilharzia* (*S. japonicum* type), *Rhodobilharzia* (*R. margrebovici,* type), and *Eurobilharzia* (*E. bomfordi,* type). These genera are in addition to the already recognized genera of *Bivitellobilharzia, Heterobilharzia* and *Schistosomatium* as members of this group of flukes. Restudy of the chromosomes of these species might help to evaluate such a separation. The same technique might also help solve the complexities of the taxonomy of avian schistosomes.

Studies of the chromosomal patterns among the Cestoidea have not been numerous. Most of the recent ones have originated from one laboratory, and there seems to have been no broad sampling of the Class as a whole. No records of studies on members of the Cestodaria have come to our attention, and attempts in this laboratory have not been successful in obtaining reliable results. Among the Cestoda only one of the 9 commonly recognized orders, the Cyclophyllidea, seems to have been examined for chromosome numbers. Among the cyclophyllideans the family Hymenolepididae is represented by records from 4 genera (8 species). These indicate that in *Diorchis* (*ralli* and *reynoldsi*) and in *Protogynella* (*blarinae*), the diploid number of chromosomes is *10*. One species of *Hymenolepis* (*H. fraterna*) has *10* chromosomes but in 3 other species (*H. ananthocephalus, H. diminuta,* and *H. serpentulus*) the number is *12*. Twelve chromosomes are also present in an unidentified species of *Aploparaksis*. *H. serpentulus* is represented by two sub-races (*sturni* and *turdi*), each with the same number of chromosomes (*12*), but the two sets of chromosomes show such consistent and easily recognizable differences that the races can be readily separated on the basis of cytological grounds alone, without reference to collection records. The Hymenolepididae, while not having a common basic number of chromosomes, are cytologically quite uniform. The same may not be said for the Dilepididae. For example, *Dipylidium* (*caninum*) has the diploid number of *10* chromosomes, *Liga* (*brasiliensis*) has *14* chromosomes, and unnamed species of *Anonchotaenia* and *Choanotaenia* each have *16* chromosomes. Among the Anoplocephalidae, *Avitellina* (*centripunctata*) has *8* somatic chromosomes, *Oochoristica* has *10*, and *Moniezia expansa* and *M. planissima* have *12*. For the Taeniidae records of $2n = 16$ for *Hydatigera* (*taeniaeformis*) and $2n = 20$ for *Taeniarhynchus* (*saginatus*) and *Taenia* (*serrata = pisiformis*) are found, although some investigators, including the present author, find only *16* chromosomes in the two latter species. Among the Nematotaeniidae, two races of *Baerietta desmognathi* have been studied, one race having $2n = 8$ and the other $2n = 16$. This difference may be an example of polyploidy but examination of a greater series of specimens would be necessary before any definite statement should be made.

It would appear from the above records that the Cestoda do not show any completely definite taxonomic pattern of chromosomal numbers above the species level. Perhaps such evidence would be forthcoming following a broader sampling among the Cestodaria and the Cestoda. We believe, on other grounds, that the Cyclophyllidea are probably the most specialized of the Cestoda, but study of chromosome numbers, structure, or behavior, has not as yet seemingly substantiated such a conclusion.

Turning to the Acanthocephala, we again find very few records of the examination of the chromosomal components of the various species. Apparently only 5 species belonging to 3 genera have been studied: *Macracanthorhynchus* (*hirudinaceous*) of the Archiacanthocephala, and *Echinorhynchus* (*acus, haeruca,* and *polymorphus*) and *Pomphorhynchus* (*proteus*) of the Palaeacanthocephala. In *Macracanthorynchus* the diploid number is *6*, and strong evidence seems to indicate that the male is heterogametic and that an X–Y pair of heterochromosomes is present. In the female the $2X$ condition seems to be present (Jones & Ward, 1950). Earlier reports of other than 6 chromosomes being characteristic of this species may be due to faulty technique or to mis-identification of the worms. *Pomphorhynchus* seems to have *8* somatic chromosomes while the 3 *Echinorhynchus* species show *16*. This

definite ratio relationship between members of the Echinorhynchidae may or may not be of phylogenetic importance, but definitely does support the separation of *Pomphorhynchus* from *Echinorhynchus,* a separation which some authorities have questioned on purely morphological grounds. It is interesting to note that in the only genus in which more than one species has been studied there is a common chromosome number. Similar examples may appear in other genera, with a broader sampling. Such constancy would not be surprising in view of the known constancy of nuclear numbers among certain of the Acanthocephala. No evidence of the presence of recognizable heterochromosomes among the Palaeacanthocephala has been presented as far as can be determined.

It would seem, on the basis of the evidence at hand, that while constancy in chromosome numbers may seem to occur within a genus, far too few records are available for making any general statement. The finding of heteromorphic conditions in one species of the Acanthocephala should encourage search for similar phenomena in other members of the phylum. As far as the actual chromosome numbers are concerned, one can only say that the low numbers reported are in accord with the high specialization of the Phylum, but do not give recognizable clues as to possible relationships, either within groups above the species level, or as to possible derivation from other phyla.

Work on the Protozoa, both free-living and parasitic, has been quite intensive, and in the past few years a great number of records have been presented. No attempt will be made to discuss all of the results, but a sampling from studies on parasitic types will indicate the general trend of the available evidence.

The Protozoa are usually divided taxonomically into four subphyla: Mastigophora, Sarcodina, Sporozoa, and Ciliophora. Examples from these groups will be selected for consideration. Among the Mastigophora, parasitic forms are normally present only in the Class Zoomastigophorea, especially in the Orders Rhizomastigida, Protomastigida, Polymastigida, Trichomonadida, and Hypermastigida. Among the Protomastigida we find examples from the Trypanosomidae (*Leishmania* and *Trypanosoma*). *Leishmania* is represented by *L. donovani* and *L. tropica,* with the chromosome number of *6* in each case, while *Trypanosoma* is represented by records from 18 species, the great majority of which have *6* chromosomes, although counts of *5, 7, 8,* and *10* have been reported. In the Trypanosomidae the basic number would seem to be *6,* and variations from that number are quite limited. The chromosomes are usually individualized, and consistent differences in size can be recognized, but neither form nor size is standardized throughout either of the genera studied.

When one turns to the Polymastigida, examples from the Pyrsonymphidae (*Notila, Oxymonas,* and *Saccinobaculus*) have been studied. *Notila proteus* has a haploid complement of *14* chromosomes, *Oxymonas doroaxostylus* has 25 ±, *Saccinobaculus ambloaxostylus* has 30 ±, and *S. lata* has 25 ±. These are probably primitive forms and have a relatively large number of small, undifferentiated chromosomes as compared to other members of the Order. In the case of members of the Tetramitidae, such as *Tetramitus rostratus,* the haploid number is reduced to *10* or *12,* with some structural differentiation present. Among the Retortamonadidae, with *Chilomastix gallinarum* possessing a haploid number of *5* chromosomes, and *R. intestinalis* having *6,* we find still further reduction in number and a greater degree of differentiation among the individual chromosomes.

In the Trichomonadida the Family Trichomonadidae has at least 11 members of the Genus *Trichomonas* for which the chromosome number has been recorded. The numbers vary from *3* in *Trichomonas buccalis* to *4* in *T. vaginalis,* to *5* in *T. eberthi, T. gallinarum,* and ? *T. hominis,* to *6* in *T. augusta, T. batrachorum, T. caviae, T. gallinae, T. hominis,* and *T. prowazeki,* and to *8* in *T. aulacodi.* It has been suggested that the number is probably *6* in all species, and that the variations are perhaps due to difficulties in interpreting the results of different techniques.

Among the Hypermastigida there are 2 families (Trichonymphidae and Holomastigotoididae) that have been studied for their chromosome numbers, primarily by Cleveland and his students. These forms, like the Pyrsonymphidae, are mainly parasitic in termites and roaches. The Trichonymphidae, as represented by *Trichonympha,* shows 26 species studied, all probably with the haploid number of chromosomes as *24,* although counts as low as *8* have been reported for *T. serbica* and *14–16* for *T. chattoni.* Other genera of this family, such as *Deltotrichonympha* (*D. numidica* with *26*), *Eucomonympha* (*E. imla* with *25*), *Barbulonympha* (*B. ufalula* with *26, B. laurabuda* with *20, B. estaboga* with *16,* and *B. wenyoni* with *12*), *Leptospironympha* (*L. eupora* and *L. wachula* with *10*), *Rhynchonympha* (*R. tarda* with *10*), and *Urinympha* (*U. talea* with *10*) all show varying stages in the evolution of gametogenesis although no marked interrelationship is indicated in terms of the numbers of chromosomes present (for details see Cleveland, 1957). Among the Holomastigotoididae, *Holomastigotoides* spp. (*bengalensis, diversa, hemogymnium,* and *tusitola*) have haploid numbers of *2, 3,* or *4,* while *Spirotrichonympha* spp. (*bispira* and *polygera*) have only *2* chromosomes. An exception seems to be *S. pyriformis* where the number is *5.* If the general concept of reduction in the number of chromosomes within a group is valid, then the Holomastigotoididae should be more specialized than the Trichonymphidae, and such seems to be the case as judged by other criteria.

The Subphylum Sarcodina has a number of parasitic species of the Class Rhizopoda whose chromosome numbers have been determined. Most of these belong to the Subclass Amoebaea and the Order Amoebida, with the Family Endamoebidae containing the truly parasitic and the entocommensal forms while the Family Amoebidae contains some ectoparasitic species. *Hydramoeba hydroxena,* ectoparasitic on hydras, has *8* chromosomes. The truly parasitic amoebae, *Entamoeba coli* with *6* chromosomes, *E. gingivalis* with *5* or *6* chromosomes, *E. muris* and *E. blattae* with *8* chromosomes, *Dientamoeba fragilis* with *4* chromosomes, and *Endolimax nana* with *10* chromosomes, do not show strong generic constancy of chromosome numbers. If constancy of numbers is a truly generic characteristic, the difference between *E. coli* with *6* and *E. blattae* with *8* chromosomes might add further evidence toward establishing the already recognized differences between *Entamoeba* (*coli*) and *Endamoeba* (*blattae*). Such evidence does not seem valid, since it would then place *E. muris* with *E. blattae* while morphologically *E. muris* more nearly resembles *E. coli.*

In the case of the Subphylum Sporozoa, a very heterogeneous and perhaps a very artificial grouping of very small forms, natural relationships are difficult to determine at the best, but some homogeneity seems to be present in some of the component subgroups. If we first examine the Telosporidea made up of the gregarines, the coccidians, and the haemosporidians, we find among the Gregarinida (Monocystidae: Acephalina) such forms as *Urospora lagidis* with *4* chromosomes, and *Zygo-*

soma globosum with *6* chromosomes. In the Gregarinidae (Cephalina), *Gregarina blattarum* has *3* chromosomes while in the Stylocephalidae (Cephalina), *Stylocephalus longicollis* has *4*. Do the acephalines give rise to or descend from the cephalines? Chromosome numbers, too few to be really indicative, seem to point to one answer, other types of evidence toward the other. In the Coccidiida we find the Adeleidae as represented by *Klossia loossi* with *4* chromosomes, by *Adelina cryptocerci* with *8* chromosomes, and *A. deronis* with *10* such bodies. Among the Eimeridae only *2* chromosomes are present in *Eimeria tenella, E. bovis,* and *Isospora felis.* Too few species have been examined, but all of those that have been studied tend to show low numbers of chromosomes and possibly are therefore to be regarded as quite removed from a probable flagellate ancestral stock which developed along the pathway of the gregarines, the coccidians, and finally the haemosporidians. The Genus *Plasmodium* (Haemosporidia: Plasmodiidae) is represented by 12 species whose chromosome numbers have been determined. There seem to be 2 quite definite groups of these species, based on their chromosomal characteristics. *Plasmodium vivax, P. ovale, P. malariae,* and *P. falciparum* (all from man), and *P. knowlesi* (from monkeys) have the haploid number of *2* unequally sized chromosomes, while *P. cynomolgi* (from monkeys) has 4 (2 large and 2 small bodies). *P. berghei* (from rats), *P. lophurae* (from ducks), *P. relictum* (from sparrows), *P. cathemerium* (from canaries), *P. gallinaceum* (from quail), and *P. floridense* (from lizards) all have the haploid number of *2* similarly sized chromosomes. *P. inui* (from monkeys) has *4* small round chromosomes, all of the same size (See Bano, 1958, and Wolcott, 1957, for details). One might speculate as to whether the haemosporidians originated from the haemogregarines of invertebrates, adopted cold-blooded vertebrates as alternate hosts, and eventually found themselves alternating between an invertebrate host and a warm-blooded host, all this with a reduction in chromosome number (?specialization), and the inclusion of periods of existence within erythrocytes as well as free-living stages in the blood stream in addition to the time spent in tissue cells. This must remain speculation as far as evidence from the chromosome aspect is concerned.

Records of studies on the other classes of the Sporozoa, such as the Cnidosporidea and the Acnidosporidea, are few and indicate only that the chromosome numbers are low (*2* to *4*) and give no clues as to their possible relationships beyond the species level. Among the Cnidosporidea we find such myxosporidians as *Chloromyxum leydigi* (with *2* chromosomes), *Ceratomyxa blennius* (with *2* chromosomes), *Myxidium gasterostei* and *M. serotinum* (each with *2* chromosomes), *Myxobolus guyenoti* and *M. dermatobius* (each with *2* chromosomes), *Myxosoma catostomi* (with *2* chromosomes), *Guyenotia sphaerulosa* (with *2* chromosomes), and *Thelohanellus catlae* (with *4* chromosomes). Only one record of a microsporidian (*Nosema notabilis,* with *2* chromosomes) has been brought to our attention. No records from the Acnidosporidea are included.

The Subphylum Ciliophora, perhaps in many ways the most specialized of the Protozoa and yet in many ways still very primitive, has not been satisfactorily related to the other protozoan groups. Since most of the ciliates are non-parasitic, only a few forms will be mentioned. The Class Ciliatea is made up of the Protociliatia and the Euciliatia. The Protociliatia are primarily parasites of amphibia, and are represented by records from 4 genera of the Family Opalinidae: *Zelleriella* (with 5 species, each with *24* diploid chromosomes, and 1 species with *38* ± diploid chro-

mosomes), *Protoopalina* (1 species with *6* ± diploid chromosomes), *Opalina* (1 species with *10* diploid chromosomes), and *Cepedea* (1 species with *6* to *8* diploid chromosomes). In all of these cases the chromosomes show consistent variations in size, shape, and method of development within a species and, at least in most *Zelleriella* species, a generic constancy in numbers. *Zelleriella* and *Protoopalina* species have only *2* nuclei, while *Opalina* and *Cepedea* species are multinucleate. There are a number of additional records (mostly made before 1934) which have not been included because of the type of material upon which they were based (poor preservation), or because of the nature of the technique employed. Recent studies have indicated that many of the so-called "chromosomes" were not composed of chromatin, but no accurate counts of the true chromosomes have been recorded in such reports. On the basis of the limited records of chromosome numbers, no phylogenetic series within the opalinids can be recognized, neither can one confirm nor dispute the possible derivation of the protociliates from the hypermastigotes; and certainly no connecting link with the Euciliatia can be demonstrated.

Most of the Euciliatia (Holotrichida, Spirotrichida, Chonotrichida, and Peritrichida) are free-living forms, but records of parasitic species among the first two orders are available for study, although very few in number. Among the Holotrichida records for only *2* species of the Genus *Conchophthirius* seem to be available. *C. anadontae* has *12* chromosomes and *C. mytili* has *16*. Although parasitic forms are somewhat more numerous among the spirotrichida (Heterotrichina), we again find only a few records of chromosome counts. *Boveria subcylindrica* has *4* chromosomes while *B. zenkevitchi* has but *2*, in each case there are 2 sizes of chromosomes. *Nyctotherus ovalis* (from the cockroach) has *4* unequal chromosomes while *N. cordiformis* (from various amphibians) has *12*, all apparently of equal size. While the number of parasitic species is far too few for comparison, reference to a considerably larger number of records from free-living forms indicates that polyploidy is common, and the presence of similar differences in number of chromosomes among the parasitic members of the same genus may point to the possibility of the same interpretation. The Spirotrichida (Oligotrichina), while mainly parasitic and modified structurally for such an existence, seemingly retain the primitive condition of having a large number of very small chromosomes, as indeed do the parasitic holotrichs. As examples we find that *Spirodinium equi* has *16* to *18* small chromosomes and *Tetratoxum unifasculatum* (also from horses) has *14* chromosomes. No records have come to our attention on the numbers of chromosomes present in the few parasitic examples among the Chonotrichida or among the Peritrichida. The same holds true for members of the Class Suctoria.

To summarize we may state our findings as follows:—

1. Study of the chromosome numbers, form, size, and behavior among the parasitic Protozoa indicates the value of such observations in the identification of species and perhaps some degree of relationship within genera. Interrelationships of categories of the generic level or higher, becomes quite speculative if based on chromosomal evidence alone. It is true, however, that the details of gametogenesis and of the place of haploid and diploid generations in the life-cycle picture may be of considerable taxonomic value, and chromosome studies should be continued within the phylum.
2. Study of the Acanthocephala gives clues as to relationships only at the species level, but such work as has been done promises interesting results.

3. Study of cestode material has been quite limited, and such few records as have been noted do not give any appreciable taxonomic clues, except perhaps to indicate that some present taxa are too heterogeneous to be of real validity. The recent studies by Jones are most promising.
4. Studies of trematode material have been relatively numerous, and the results point definitely toward the desirability of continued efforts in this field of research. The discovery of the presence of heterochromosomes, particularly of the fact that some forms show the female as the heterogamic sex, is of especial interest and importance. Some definite taxonomic relationships are recognizable, but since in some cases they substantiate other types of evidence used in establishing phylogenetic position and in other cases the results seem to be contradictory, further observations are in order. Perhaps some of the apparent contradictions may be eliminated as our knowledge increases. Such definitely has been the case among the Turbellaria, as mentioned in the body of this paper.
5. Study of the parasitic nematodes has given a considerable amount of valuable taxonomic information, and here again, the field is by no means exhausted.

The cytologist has a broad field of endeavor in the study of parasitic forms. With the newer techniques and the newer optical equipment now available, new knowledge of the physiology, morphology and phylogenetic relationships of such parasitic forms as those discussed is just at the threshold of a whole vista of new concepts and new interpretations.

Explanation of Classification

The classification of the nematodes is based mainly on that of Chitwood; that of the Turbellaria, mainly on that of Hyman; that of the trematodes, mainly on that of Chandler; that of the cestodes, mainly that of Wardle and McCleod; that of the Acanthocephala, on that of Van Cleave; and that of the Protozoa, on that of Hall.

Chromosome Numbers and Authorities

Classification of Parasite	*Parasite*	*Number*	*Authority*
NEMATODA			
Chromadorata			
Desmadoridae	*Spirina parasitifera*	7	Cobb, 1928
Rhabditata			
Rhabditidae	*Rhabditis aspera*	7	Walton, 1940
	R. monhysterii	7	Nignon, 1947
	R. pellio	7	Walton, 1940
	R. aberrans	9	Kruger, 1913, Walton, 1940
Rhabdiasidae	*Rhabdias briggsiae*	6	Nignon & Dougherty, 1949
	R. bufonis	6	Schleip, 1911
	R. elegans	6	Nignon & Dougherty, 1949
	R. fülleborni	6	Dreyfus, 1937
Strongyloididae	*Strongyloides papillosus*	3	Chang & Graham, 1957
	S. ratti	3	Nignon & Roman, 1952
Ascaridata			
Ascaroidea			
Ascaridae	*Ascaris lumbricoides* (= ? *suis*)	24	Homedes, 1933
	Neoascaris vitulorum	9	Homedes, 1933
	Ophidascaris filaria	7	Walton, this paper
	Parascaris equorum, var. 1	9	Li, 1937
	P. equorum, var. 2	6	Li, 1934
	P. equorum, var. 3	3	Walton, 1924
	P. equorum, var. 4	2	Walton, 1924
	P. equorum, var. 5	1	Walton, 1924
	Toxacara cati	9	Walton, 1924
	T. canis	18	Walton, 1924
	T. culpis	12	Walton, 1924

CHROMOSOME NUMBERS AND AUTHORITIES (*Continued*)

Classification of Parasite	*Parasite*	*Number*	*Authority*
Anisakidae	*Contracaecum clavatum*	12	Walton, 1940
	C. incurvum	21	Goodrich, 1916
	C. spiculigerum	5	Walton, 1924
Kathlaniidae	*Cruzia tentaculata*	6	Walton, 1924
Heterakidae	*Ascaridia galli*	5	Walton, 1940
	Heterakis dispar	5	Gulick, 1911, Walton, 1940
	H. gallinae	5	Gulick, 1911, Walton, 1940
	H. papillosa	5	Gulick, 1911, Walton, 1940
	H. sp?	5	Walton, 1924
	H. spumosa	? 6	Walton, 1924
Oxyuroidea			
Oxyuridae	*Passalurus ambiguus*	4	Meves, 1920
	Syphacia obvelata	8	Walton, 1924
Trichurata			
Trichosomoididae	*Trichosomoides crassicauda*	4	Walton, 1924
Strongylata			
Heligmosomidae	*Nematospira turgida*	6	Walton, 1924
Strongylidae	*Cyclostomum tetracanthum*	6	Walton, 1940
	Strongylus edentatus	6	Kühtz, 1913, Walton, 1940
	S. equinum	6	Kühtz, 1913, Walton, 1940
	S. vulgaris	6	Kühtz, 1913, Walton, 1940
	Stephanurus dentatus	6	Tromba & Stecke, 1957
Metastrongylidae	*Dictyocaulus filaria*	6	Struckmann, 1906, Walton, 1940
	D. viviparous	6	Walton, 1940
	Filaroides mustelarum	8	Carnoy, 1896, Walton, 1940
	Metastrongylus elongatus	6	Gulick, 1911, Walton, 1940
Trichostrongylidae	*Haemonchus contortus*	6	Bremner, 1955
	Trichostrongylus tenuis	6	Gulick, 1911, Walton, 1940
Spirurata			
Spiruroidea			
Acuariidae	*Dispharynx spiralis*	6	Walton, 1924
Camallanidae	*Camallanus lacustris*	6	Walton, 1940
Physalopteridae	*Physaloptera turgida*	5	Walton, 1924
	Proleptus robustus	8	Carnoy, 1886, Walton, 1940
Rhabdochonidae	*Cystidicola farionis*	6	Mulsow, 1912
Spiruridae	*Protospirura muris*	5	Walton, 1924
	Spirura talpae	8	Carnoy, 1886, Walton, 1940
Filarioidea			
Setariidae	*Setaria equina*	6	Meves, 1915
NEMATOMORPHA			
Gordioidea			
Gordiidae	*Gordius* (all spp?)	2 (?4)	Svábenik, 1909, Meyer, 1913, Muhldorf, 1914, Vejdovsky, 1912, Camerano, 1890
Chordodidae	*Paragordius varius*	7	Montgomery, 1904
TURBELLARIA			
Acoela			
Convolutidae	*Aphanostoma diversicolor*	20–30	Ruebush, 1938
	Convoluta sp?	20–30	Ruebush, 1938
	Polychoerus caudatus	31	Gardiner, 1898
Alloeocoela			
Bothrioplanidae	*Bothrioplana semperi*	10–15	Pennypacker, 1954
Plagiostomidae	*Hydrolimax grisea*	10	Pennypacker, 1954
	Plagiostomum stellatum	5	Ruebush, 1938, Pennypacker, 1954
Prorhynchidae	*Geocentrophora applanatus*	10	Ruebush, 1938, Pennypacker, 1954
	Prorhynchus stagnalis	10	Ruebush, 1938, Pennypacker, 1954
Pseudostomidae	*Pseudostomum caecum*	8–10	Jones, 1943, Pennypacker, 1954
	P. sp?	4–5	Ruebush, 1938, Pennypacker, 1954
Monocelididae	*Monocelis fusca*	3	Ruebush, 1938, Pennypacker, 1954

CHROMOSOME NUMBERS AND AUTHORITIES (*Continued*)

Classification of Parasite	*Parasite*	*Number*	*Authority*
Tricladida			
Planariidae	*Curtisia foremanni*	6	Pennypacker, 1954
	Polycelis tenuis	6	Melander, 1950, Lepori, 1953
	P. nigra	18	Lepori, 1953
	Dugesia benazzi	8 & 16	Benazzi-Lentati & Narchi, 1950
	D. gonocephala	8 & 16	Schleip, 1907, Benazzi-Lentati, 1949
	D. alpina	12	Rappeport, 1915, Benazzi-Lentati, 1949
	D. sp?	24	Ruebush, 1938
	Planaria polychroa	8	Mattieson, 1904, Ruebush, 1938
	P. torva	8	Mattieson, 1904, Ruebush, 1938
Dendrocoelidae	*Dendrocoelum lacteum*	7 & 8	Pennypacker, 1954
	D. infernale	16	Aeppli, 1951, Pennypacker, 1954
	Procotyla fluviatilis	7	Pennypacker, 1954
	Bdellocephala brunnea	10	Momma, 1953
Bdellouridae	*Bdelloura candida*	6–12	Pennypacker, 1954
Procerodidae	*Procerodes gerlachei*	6	Böhmig, 1905, Ruebush, 1938
Polycladida			
Euryleptidae	*Cycloporus papillosus*	8	Francotte, 1898, Ruebush, 1938
	Oligocladus auritus	8	Francotte, 1897, Ruebush, 1938
	Prosthecereaus vittatus	?6	Gerard, 1901, Ruebush, 1938
Leptoplanidae	*Leptoplana tremellaris*	8	Francotte, 1898, Ruebush, 1938
Prothiostomidae	*Prosthiostomum siphunculus*	8	Francotte, 1898, Ruebush, 1938
Pseudoceridae	*Thysanozoon brocchi*	9	Schockaert, 1905, Ruebush, 1938
Stylochidae	*Stylochus pilidium*	9	Gerard, 1901, Ruebush, 1938
	Eustylochus ellipticus	10	Von Name, 1899, Ruebush, 1938
Planoceridae	*Planocera inquilina*	10	Patterson & Wieman, 1912, Ruebush, 1938
	P. nebulosa	10	Von Name, 1899, Ruebush, 1938
Rhabdocoela			
Catenulidae	*Fuhrmannia* sp? (= *Suomina* sp?)	16	Ruebush, 1938
	Catenula virginiana	20	Ruebush, 1938
	Rhyncoscolex simplex	20	Ruebush, 1938
	Stenostomum grandi	20	Ruebush, 1938
	S. sp?	20	Ruebush, 1938
Temnocephalidae	*Temnocephalus canis*	8	Ruebush, 1938
Typhloplanidae	*Promesostoma marmoratum*	6	Ruebush, 1938
	Bothriomesostoma personatum	5	Ruebush, 1938
	B. essenii	5	Papi, 1950
	Byrsophlebs sp?	4	Ruebush, 1938
	Mesostoma rhynchotum	8	Valkanov, 1938
	M. ehrenbergii (Europe)	5	Papi, 1950
	M. chr. wardi (U.S.A.)	4	Husted, et al., 1939
	M. benazzii	4	Papi, 1950
	M. lingua	2 & 3	Ruebush, 1938
	Castrata subsala	3	Papi, 1950
	C. virginiana	3	Ruebush, 1938
	C. spp?	3	Ruebush, 1938
	Typhloplana viridata	3	Ruebush, 1938
	Trigonostomum lillei	3	Ruebush, 1938
	Protoplanella sp?	3	Ruebush, 1938
	P. sp?	2	Ruebush, 1938
	Opistomum pallidum	2	Ruebush, 1938, Papi, 1952, 1953
	O. sp?	4	Ruebush, 1938
	Krumbachia minuta	2	Ruebush, 1938
	K. virginiana	2	Ruebush, 1938
	Solenopharynx sp?	2	Ruebush, 1938
	Amphibolella virginiana	2	Ruebush, 1938
	Phaenocora kepneri	2	Ruebush, 1938
	P. lutheri	2	Ruebush, 1938
	P. virginiana	2	Ruebush, 1938
	P. sp?	2	Ruebush, 1938
	Rhynchomesostoma rostratum	2 & 3	Volkanov, 1938, Papi, 1950
Kalyptorhynchidae	*Polycystis goettei*	8	Ruebush, 1938
	Microkalyptorhynchus virginianus	3	Ruebush, 1938
	Acrorhynchus reprobatus	2	Ruebush, 1938
	Gyratrix hermaphroditicus	2	Ruebush, 1938
Provorticidae	*Provortex affinis*	3	Ruebush, 1938
	P. sp?	3	Ruebush, 1938

CHROMOSOME NUMBERS AND AUTHORITIES (*Continued*)

Classification of Parasite	*Parasite*	*Number*	*Authority*
Graffillidae	*Paravortex cardii*	2	Hallez, 1908, Ruebush, 1938
	P. gemellipara	4	Ball, 1916, Ruebush, 1938
Macrostomidae	*Macrostomum hystrix*	2	Ferguson, 1940
	M. viride	2	Ferguson, 1940
	M. beaufortensis	3	Ferguson, 1940
	M. fuba	3	Ferguson, 1940
	M. virginianum	3	Ferguson, 1940
	M. hustedi	6	Jones, 1944
	M. bispiralis	8	Ferguson, 1940
	M. kepneri	9	Ferguson, 1940
Dalyelliidae	*Castrella truncata*	2	Ruebush, 1938
	Dalyellia abursalis	2	Ruebush, 1938
	D. armigera	2	Ruebush, 1938
	D. triangulata	2	Ruebush, 1938
	D. virginiana	2	Ruebush, 1938
	D. viridis	2	Ruebush, 1938
	D. (4 spp?)	2	Ruebush, 1938
TREMATODA			
Monogenea			
Polystomidae	*Gyrodactylus elegans*	6	Gille, 1914
	Polystoma integerrimum	4	Goldschmidt, 1902
Digenea			
Paramphistomatidae	*Gigantocotyle bathocotyle*	6	Willmott, 1950
	Gastrothylax crumenifer	7	Dhingra, 1955
	Zygocotyle lunata	7	Willey & Goodman, 1951
	Colylophoron elongatum	8	Dhingra, 1955
	Diplodiscus temperatus	8 & 9	Cary, 1909, Britt, 1947, Van der Woude, 1954
	Heronimus chelydrae	10	Guilford, 1955
Heterophyidae	*Cryptocotyle lingua*	6	Cable, 1931, Britt, 1947
	Acetodextra amiuri	6	Perkins, 1956
Bucephalidae	*Bucephalus elegans*	6	Woodhead, 1931, Ciordia, 1956
	B. pusillus	6	Woodhead, 1931, Ciordia, 1956
	Rhipidocotyle papillosum	6	Ciordia, 1956
Fasciolidae	*Fasciola hepatica*	6	Schellenberg, 1911, Yosufzai, 1953
Zoogonidae	*Zoogonus mirus*	6	Wassermann, 1913, Anderson 1935
Notocotylidae	*Notocotylus flamentis*	7	Ciordia, 1950
Schistosomatidae	*Schistosomatium douthitti*	7	Nez, 1954, Short, 1957, Woodhead, 1957
	Schistosoma haematobium	8	Lindner, 1914, Markell, 1943
	S. japonica	8	Anderson, 1935, Ikada & Makino, 1936
	S. mansoni	8	Anderson, 1935, Niyamasena, 1940
	Ornithobilharzia canaliculata	8	Short & Menzell, 1957
Allocreadiidae	*Allocreadium isoporum*	8	Britt, 1947
	Crepidostomum serpentinum	8	Britt, 1947
	Bunodera luciopercae	7	Britt, 1947
	B. saculata	8	Britt, 1947
Rhopaliidae	*Rhopalias macracanthus*	8	Ciordia, 1949
Troglotrematidae	*Paragonimus kellicotti*	8	Chen, 1937 Cort, 1944, Britt, 1947
Gorgoderidae	*Probolitrema californiense*	6	Markell, 1943, Britt, 1947
	Gorgoderina attenuata	7	Willey & Koulish, 1950
	Gorgodera amplicava	8	Britt, 1947
	Phyllodistomum spatula	8	Dhingra, 1954
Azygiidae	*Azygia acuminata*	9	Britt, 1947
	Proterometra macrostoma	9	Anderson, 1935, Britt, 1947
Spirorchidae	*Spirorchis magnitestis*	9	Jones & Mayer, 1953
Pronocephalidae	*Macrovestibulum kepneri*	9	Jones, et al., 1946
Monorchidae	*Asymphylodera indica*	9	Dhingra, 1955
Cyclocoelidae	*Cyclocoelum bivesiculatum*	10	Dhingra, 1954
Dicrocoeliidae	*Brachycoelium salamandrae*	10	Anderson, 1935
	Dicrocoelium lanceolatum	10	Dingler, 1910, Anderson, 1935
Clinostomatidae	*Clinostomum marginatum*	10	Britt, 1947
Hemiuridae	*Halepegus occidualis*	9	Jones, 1956
	Isoparorchis eurytremum	10	Dhingra, 1954

CHROMOSOME NUMBERS AND AUTHORITIES (*Continued*)

Classification of Parasite	*Parasite*	*Number*	*Authority*
Echinostomatidae	*Parorchis acanthus*	11	Rees, 1939
Lecithodendriidae	*Acanthatrium pipistrella*	11	Britt, 1947
	Loxogenes bicolor	11	Britt, 1947
	Brandesia turgidum	9	Levy, 1913
Plagiorchiidae	*Eustomas chelydrae*	9	Britt, 1947
	Glypthelmins quieta	9	Britt, 1947
	Plagitura salamandrae	11	Britt, 1947
	Pneumonoeces breviplexus	11	Britt, 1947
	P. medioplexus	11	Pennypacker, 1940
	P. similiplexus	11	Pennypacker, 1940
Reniferidae	*Staphylodora bascaniensis*	8	Britt, 1947
	Telorchis robustus	8	Britt, 1947
	Auridistomum chelydrae	9	Britt, 1947
	Dasymetra villicaeca	11	Britt, 1947
	Lechriorchis abduscens	11	Britt, 1947
	Natriodora verlatum	11	Britt, 1947
	Neorenifer aniarum	11	Britt, 1947
	N. drymarchon	11	Britt, 1947
	N. elongatus	11	Britt, 1947
	N. georgianus	11	Britt, 1947
	N. orula	11	Britt, 1947
	N. wardi	11	Britt, 1947
	Pneumatophilus leidyi	11	Britt, 1947
	P. variabilis	11	Britt, 1947
	Renifer ellipticus	11	Britt, 1947
	Telorchis corti	11	Britt, 1947
	T. lobosus	11	Britt, 1947
	T. medios	11	Britt, 1947
Cephalogonimidae	*Cephalogonimus americanus*	14	Britt, 1947
ACANTHOCEPHALA			
Archiacanthocephala			
Oligacantho-rhynchidae	*Macracanthorhynchus hirudinaceus*	6	Noé, 1914, Jones & Ward, 1950
Palaeacanthocephala			
Echinorhynchidae	*Pomphorhynchus proteus*	8	Von Voss, 1910
	Echinorhynchus acus	16	Hamann, 1891
	E. haeruca	16	Hamann, 1891
	E. polymorphus	16	Hamann, 1891
CESTOIDEA			
Cestoda			
Cyclophyllidea			
Hymenolepididae	*Diorchis ralli*	10	Jones, 1945
	D. reynoldsi	10	Jones, 1945
	Protogynella blarinae	10	Jones, 1945
	Hymenolepis fraterna	10	Jones, 1945
	H. anthocephalus	12	Jones, 1945
	H. diminuta	12	Jones, 1945
	H. serpentulus sturni	12	Jones, 1945
	H. serpentulus turdi	12	Jones, 1945
	Aploparaksis sp?	12	Jones, 1945
Dilepididae	*Dipylidium caninum*	10	Jones, 1945
	Anonchotaenia globata	12	Jones, 1945
	Rhabdometra similis	12	Jones, 1945
	Liga brasiliensis	14	Jones, 1945
	Anonchotaenia sp?	16	Jones, 1945
	Choanotaenia (= *Monopylidium* sp?)	16	Jones, 1945
Anoplocephalidae	*Avitellina centripunctata*	8	Gough, 1911
	Oochoristica sp?	10	Jones, 1945
	Moniezia expansa	12	Child, 1907
	M. planissima	12	Child, 1907
Taeniidae	*Taeniarhynchus taeniaeformis* (= *Hydatigera* sp.)	16	Jones & Ciordia, 1956
	T. saginatus	20	Jones, 1957
	Taenia serrata (= *pisiformis*)	16–20	Jones & Ciordia, 1956
Davaineidae	*Davainea proglottina*	18	Jones, 1951
Nematotaeniidae	*Bacrietta desmognathi*	8	Douglas, 1957
	B. desmognathi (2nd race)	16	Douglas, 1957
PROTOZOA			
Mastigophora—Zoomastigophorea			
Protomastigida			
Trypanosomidae	*Leishmania donovani*	6	Gupta, et al., 1954
	L. tropica	6	Roskin, et al., 1928
	Trypanosoma brucei	6–8	Roskin, et al., 1928

CHROMOSOME NUMBERS AND AUTHORITIES (*Continued*)

Classification of Parasite	*Parasite*	*Number*	*Authority*
	T. chagasi	5	Horta & Machado, 1911
	T. congolense	6	Vanderplank, 1944
	T. cruzi	6–?10	Noble, et al., 1953
	T. dimorphon	10	Hindle, 1909
	T. equinum	6	Hoare, 1950
	T. equiperdum	6	Ray, et al., 1955
	T. evansi	6	Roskin, et al., 1928
	T. gambiense	6	Roskin, et al., 1929
	T. lewisi	6	Wolcott, 1952
	T. pecaudi	6	Roskin, et al., 1929
	T. pseudi	6	Roskin, et al., 1929
	T. raiae	6	Robertson, 1930
	T. rhodesiense	5–6	Vanderplank, 1944, Fairbairn, 1946
	T. simiae	6	Fairbairn, et al., 1946
	T. suaura	6	Roskin, et al., 1929
	T. theileri	5	Hartman & Nöller, 1918
	T. vespertilionis	8	Gonder, 1910
Polymastigida			
Pyrsonymphidae	*Notila proteus*	14	Cleveland, 1950
	Oxymonas dorvaxostylus	25 ±	Cleveland, 1950
	Saccinobaculus lata	25 ±	Cleveland, 1950
	S. amboaxostylus	30 ±	Cleveland, 1950
Tetramitidae	*Tetramitus rostratus*	10–12	Rafalko, 1951
Retortamonadidae	*Chilomastix gallinarum*	5	McDowell, 1953
	C. intestinalis	6	McDowell, 1948
Trichomonadida			
Trichomonadidae	*Trichomonas buccalis*	3	Hinshaw, 1926
	T. vaginalis	4	Powell, 1936
	T. eberthi	5	McDowell, 1953
	T. gallinarum	5	McDowell, 1953
	T. hominis	?5–6	Bishop, 1931
	T. augusta	6	Samuels, 1941
	T. batrachorum	6	Samuels, 1957
	T. caviae	6	Nie, 1950
	T. gallinae	6	Stabler, 1941
	T. prowazeki	6	Samuels, 1955
	T. aulacodi	8	Hollande & Enjumet, 1953
Hypermastigida			
Trichonymphidae	*Trichonympha* (26 spp.)	24	Cleveland, 1949
	T. chattoni	?14–16	Duboscq & Grassé, 1927
	T. serbica	?8	Georgovitch, 1932
	Deltotrichonympha numidica	26	Duboscq & Grassé, 1943
	Eucomonympha imla	25	Cleveland, 1950
	Barbulonympha ufalula	26	Cleveland, 1953
	B. laurabuda	20	Cleveland, 1953
	B. estaboga	16	Cleveland, 1953
	B. wenyoni	12	Cleveland, 1953
	Leptospironympha eupora	10	Cleveland, 1951
	L. wachula	10	Cleveland, 1951
	Rhynchonympha tarda	10	Cleveland, 1952
	Urinympha talea	8	Cleveland, 1951
Holomastigotoid-idae	*Holomastigotoides bengalensis*	4	Chakravarty & Benerjee, 1956
	H. diversa	2–3–4	Cleveland, 1949
	H. hemogymnium	2–3	Mackinnon, 1926
	H. tusitola	2–3	Cleveland, 1949
	Spirotrichonympha pyriformis	5	Chakravarty & Benerjee, 1956
	S. bispira	2	Cleveland, 1938
	S. polygera	2	Cleveland, 1938
Sarcodina			
Rhizopoda—Amoebaea			
Amoebida			
Endamoebidae	*Endolimax nana*	10	Dobell, 1943
	Endamoeba blattae	8	Megalitsch, 1940
	Entamoeba muris	8	Wenrich, 1940
	E. coli	6	Stabler, 1932
	E. gingivalis	5–6	Stabler, 1940, Noble, 1947
	Dientamoeba fragilis	4	Wenrich, 1944
Amoebidae	*Hydramoeba hydroxena*	8	Reynolds, et al., 1929
Sporozoa—Telosporidea			
Gregarinida			
Monocystidae	*Urospora lagidis*	4	Naville, 1927
	Zygosoma globosum	6	Noble, 1938
Gregarinidae	*Gregarina blattarum*	3	Sprague, 1941
Stylocephalidae	*Stylocephalus longicollis*	4	Grell, 1940

CHROMOSOME NUMBERS AND AUTHORITIES (*Continued*)

Classification of Parasite	*Parasite*	*Number*	*Authority*
Coccidiida			
Adeleida			
Adeleidae	*Klossia loossi*	4	Husted & Ruebush, 1940
	Adelina cryptocerci	8	Yarwood, 1937
	A. deronis	10	Hauschka, 1943
Eimeriida			
Eimeriidae	*Eimeria bovis*	2	this paper
	E. tenella	2	this paper
	Isospora felis	2	this paper
Haemosporidia			
Plasmodiida			
Plasmodiidae	*Plasmodium berghei*	2	Wolcott, 1957
	P. cathemerium	2	this paper
	P. falciparum	2	Wolcott, 1957
	P. floridense	2	Wolcott, 1957
	P. gallinaceum	2	this paper
	P. knowlesi	2	Wolcott, 1957
	P. lophurae	2	this paper
	P. malariae	2	Wolcott, 1957
	P. ovale	2	Wolcott, 1957
	P. relictum	2	this paper
	P. vivax	2	Wolcott, 1957
	P. cynomolgi	4	Bano, 1958
	P. inui	4	Bano, 1958
—Cnidosporidea			
Myxosporida			
Ceratomyxidae	*Ceratomyxa blennius*	2	Noble, 1941
Chloromyxidae	*Chloromyxum leydigi*	2	Naville, 1927
Myxidiidae	*Myxidium gasterostei*	2	Noble, 1941
	M. serotinum	2	Kudo, 1943
Myxosomatidae	*Myxosoma catostomi*	2	Kudo, 1926
Myxobolidae	*Myxobolus dermatobius*	2	Kudo, 1933
	M. guyenoti	2	Kudo, 1933
Thelohanellidae	*Thelohanellus catlae*	4	Chakravarty, et al., 1948
Actinomyxida	*Guyenotia sphaerulosa*	2	Naville, 1930
Microsporida			
Nosematidae	*Nosema notabilis*	2	Kudo, 1944
Ciliophora—Ciliatea			
Protociliatia			
Opalinidae	*Zelleriella* sp?	38 ±	Chen, 1948
	Z. elliptica	24	Chen, 1948
	Z. intermedia	24	Chen, 1948
	Z. louisianensis	24	Chen, 1948
	Z. pfitznori	24	Chen, 1948
	Z. valliceps	24	Chen, 1948
	Opalina carolinensis	10	Hunter, 1955
	Cepedea dimidiata	6–8	Ivanić, 1933
	Protoopalina fasciata	6	Fantham, 1931
Euciliatia			
Holotrichida			
Isotrichidae	*Conchophthirius mytili*	16	Kidder, 1934
	C. anadontae	12	Kidder, 1934
Spirotrichida			
Heterotrichina			
Boveridae	*Boveria subcylindrica*	4	Kidder, 1934
	B. zenkevitchi	2	Levinson, 1941
Plagiostomidae	*Nyctotherus cordiformis*	12	Wichterman, 1937
	N. ovalis	4	Wichterman, 1937
Oligotrichina			
Ophryoscolecidae	*Spirodinium equi*	16–18	Davis, 1941
Cycloposthidae	*Tetratoxum unifasculatum*	14	Davis, 1941

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A MICROSPORIDIAN PARASITE OF *ANOPHELES GAMBIAE* IN LIBERIA

RICHARD M. FOX * AND JARASLAV WEISER **

In connection with a survey of wild *Anopholes gambiae* for natural infections of malaria, conducted at the Liberian Institute, Harbel, Liberia, West Africa, one of us made some thousands of midgut dissections during the period from June 1955 until April 1956 (Fox, 1957). From time to time a wild mosquito was found to be infected with a microsporidian parasite. The rate of this natural infection was not recorded but was exceedingly low.

Colonies of *A. gambiae* had been maintained at the Institute continuously for the previous 4 years without difficulty. During the summer of 1956 an outbreak of nosematosis occurred, quickly reaching nearly epizootic proportions and spreading to the colony of *A. melas*. Because infected mosquitoes are not suitable for use in malaria study, the colonies had to be destroyed. A few infected strains were kept for further study of the parasite.

The purpose of the present paper is to record the pathology of the microsporidian infection, identified as *Nosema stegomyiae*, in adult *A. gambiae* and to present certain observations on nosematosis in mosquitoes.

Nosema stegomyiae (Marchoux, Salimbeni and Simond), 1903, has reniform or comma-form spores 4–7 microns long and 2–3 microns wide. It invades the gut, muscles, air sacs, coelomic lining, neural ganglia and ovaries of adult mosquitoes, and was originally observed as a parasite of *Aedes*. *N. stegomyiae* Lutz and Splendore, 1908, described as a new species, and *N. anophelis* Kudo, 1924, are synonyms.

Two other microsporidia parasitize mosquitoes: *Thelohania grassi* Nicholson, 1921, had earlier been recorded but not described by Grassi (1901), Ross (1906), Stephens and Christophers (1908); *Plistophora culicis* Weiser, 1947, with spores 5 microns long and plasmodia 15 microns in diameter, has been found and fully detailed by Garnham (1956) and Canning (1957). Both these species sometimes invade the larvae. One of us (JW) is preparing a monographic revision of the microsporidia.

MATERIALS AND METHODS

Adult *A. gambiae* infected with *N. stegomyiae* were fixed in Bouin's fluid, stained either with Giemsa or with Heidenhain's iron haematoxylin and studied in serial section. Mosquitoes dissected in physiologic saline solution afforded fresh, unfixed material for study.

OBSERVATIONS

Pathology of the infection in Anopheles gambiae

Infections in the adult mosquito cannot ordinarily be recognized by behavior or by external characteristics, but the presence of *N. stegomyiae* is immediately evident

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upon dissection. The infected midgut, in particular, is readily recognized even with the naked eye because of its opaque, chalk-white color, its often irregular shape and its loose texture suggesting disintegration. When examined in saline at about 500 × magnification with transmitted light, the normally translucent midgut tissue is seen to be obscured by masses of denser spores. In heavy infections the parasites can be so numerous that none of the midgut tissue can be seen. Because the midgut is invaded early in the course of the infection, its dissection affords the most convenient procedure for diagnosis of nosematosis. In the sectional material studied it was found that all organs and tissues were eventually invaded, except the tracheae, the air sacs and the coelomic lumina.

Alimentary tract: Some mosquitoes were found to be parasitized only in the mouth region, and this site was involved in all specimens studied, however widely parasitized. The first part of the alimentary system infected by the microsporidian was the epithelium of the esophageal pump. From this focus, diffuse groups of schizonts found their way into the esophageal and proventricular epithelium (Fig. 1). The midgut soon became heavily infected; spores and schizonts first were found

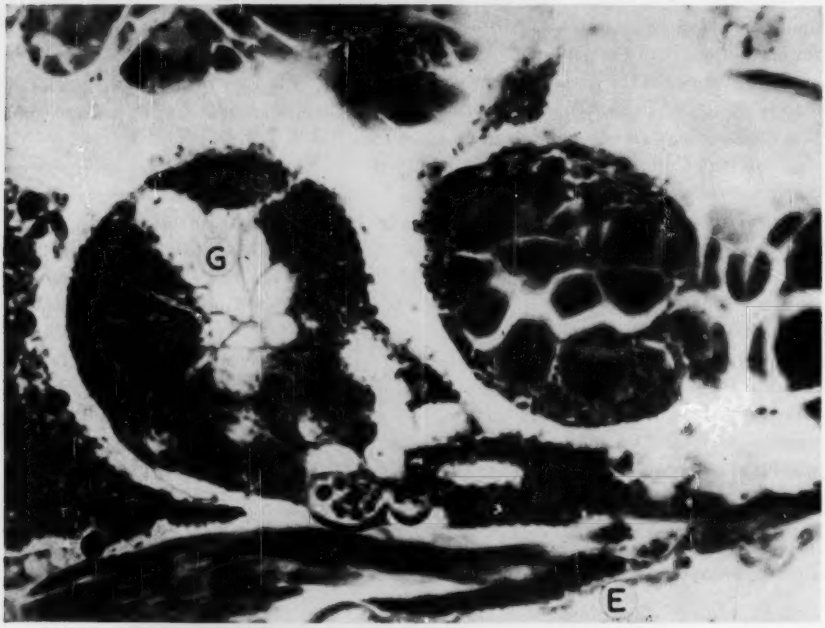

FIGURE 1. Section through prothorax of *Anopheles gambiae* showing infection of *Nosema stegomyiae* in the salivary gland (G), the fat body sheath and a part of the oesophagus (E).

in the inner epithelium. Infected cells were pressed into the body cavity of the mosquito (Figs. 2, 3) and after rupturing the muscular layer of the gut as the infection progressed, they broke off into the body cavity and floated there as pseudocysts. As more and more of the epithelial cells became infected, that layer disintegrated (Fig. 4), the remnants being loosely confined by the remnants of the muscle

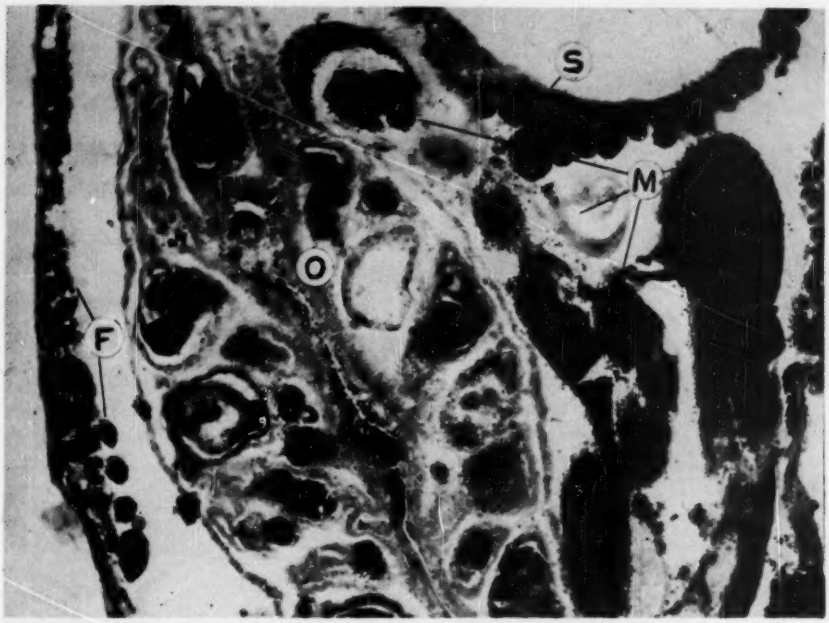

FIGURE 2. Section through abdomen showing infected ovary (O) with many of the eggs destroyed, infection in the malpighian tubules (M), in the midgut wall (S) and in the fat body (F).

FIGURE 3. Section through abdomen of a non-infected female *A. gambiae* showing structures as in figure 2 for a healthy mosquito.

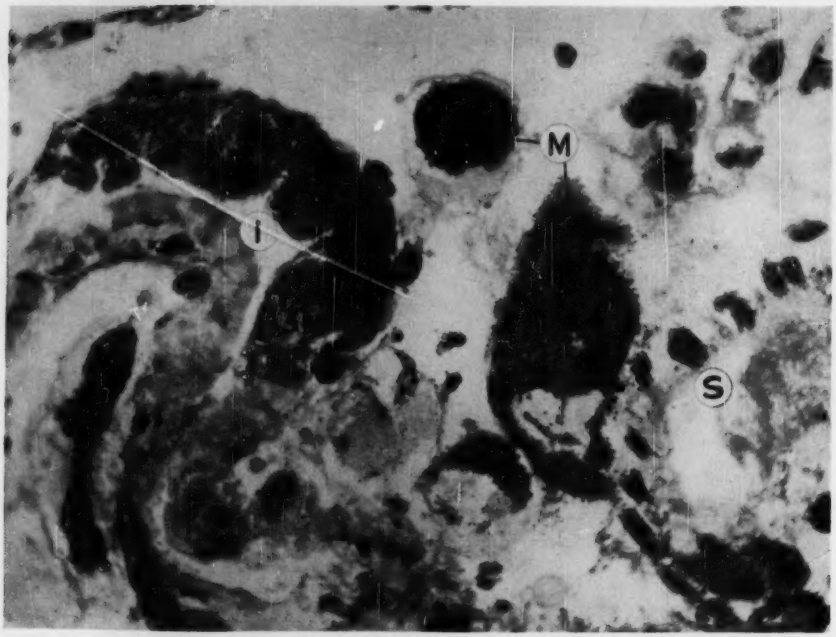

FIGURE 4. Section through the abdomen of an infected female mosquito showing microsporidial invasion in the malpighian tubules (M) and the proctodeum (I). The disintegrated midgut wall appears at the right (S).

layers. While this muscle tissue was not itself ordinarily infected, it tended to degenerate in the absence of the nutrition afforded by healthy epithelium. The peritrophic membrane with groups of yeast cells, found in the healthy individual, was not found in infected mosquitoes. Remnants of blood cells in clusters in the gut lumen frequently were arranged in several distinct layers which doubtless indicated the number of feedings taken by the mosquito after infection with *Nosema*.

When infected cells burst into the body cavity, the spores were phagocytized by lymphocytes normally present in the hemolymph. The hindgut soon became heavily infected, but thanks to the presence of the chitinous membrane, the tissue cells distended by spores were held in position (Fig. 4). The feces were heavily contaminated with spores and ultimately the entire fecal mass was composed only of spores.

Malpighian tubules: These were almost always heavily infected. Cells of the secretory part were filled out with spores but without hyperplasy (Figs. 2, 3, 4). In some cases not all tubules were infected to the same degree and entirely healthy parts could be found even in heavy infections.

Salivary glands: The parasite apparently entered the salivary duct early in the course of the infection, and thence spread to the gland tissue. Masses of microsporidia distended whole groups of secretory cells surrounding a single lumen (Fig. 1), but those cells escaping infection did not appear to be affected or deformed in any way by the parasite or its secretions.

Respiratory system: The tracheal matrices and the air sacs were not found to be infected. In a few cases spores were found clinging to the outer layer of the sac

wall, but these probably derived from pseudocysts which burst in the coelom.

Circulatory system and coelom: Development of the microsporidian in the body cavity without a cellular substrate was not seen. The heart muscles frequently were found infected. Of the haemocytes, only lymphocytes were involved in phagocytosis of those stages freed into the body cavity. Phagocytosed elements were not destroyed but remained alive within the phagocyte. In this situation the schizonts continued their development. Ultimately the phagocyte became a pseudocyst containing a mass of spores. In sectioned material no changes in the shape of cells nor in number of nuclei of the parasite were noted, nor was any change in the number of lymphocytes found. Giant cells, sometimes found in microsporidian infections, were not present. Atretization, melanization or other forms of destruction of the parasite in the mosquito tissues were not found.

Fat body: Throughout the fatty tissue, cells were found infected (Figs. 1, 2, 3), often with heavily infected areas located near areas infected only with the first wave of schizonts.

Musculature: In the big active muscles of the head and thorax, diffuse infected fibers were found with the parasite infiltrated between parallel fibrillae (Fig. 5).

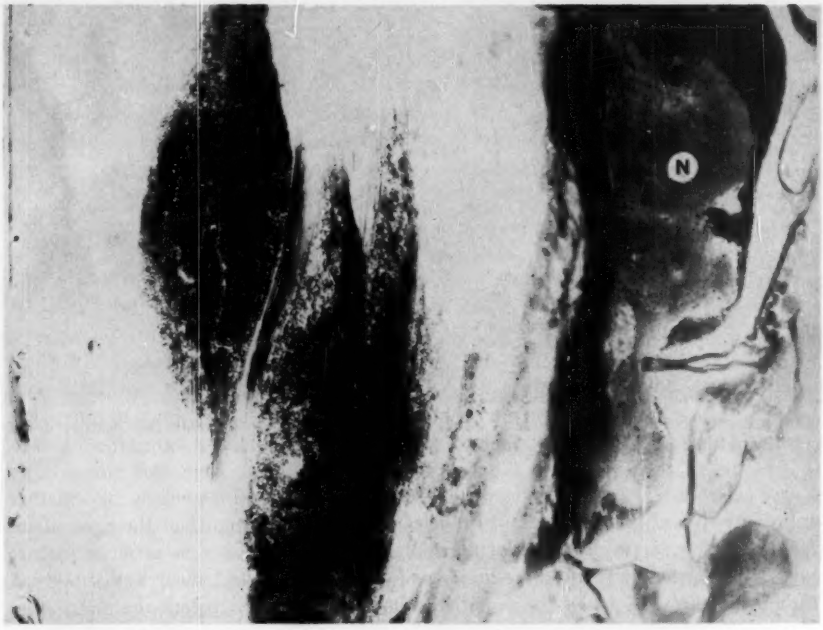

FIGURE 5. Section through mesothorax of *A. gambiae* showing the thoracic ganglion (N) and thoracic muscles infected by *N. stegomyiae*.

Here longitudinal lacunae were formed in which spores accumulated. Total destruction of the musculature was not found. The infection was commonly found in the muscles of the abdominal segments and in fibers holding the various organs in place, with these tissues altered by the spore masses.

Nervous system: All major ganglia of the body eventually became infected. The invasion as spread from the fatty sheath of the ganglia differed from that as spread from the endothelial sheath. In the dorsal ganglion of the mosquito, microsporidia were found only in the inner fibrous part and the ganglial cells proper were not affected. In the thoracic ganglia (Fig. 5), on the other hand, the ganglial cells were invaded before the pulp. Neuroreceptive organs were not found to be infected.

Gonads: These received the infection late in the cycle. In massive infections, schizonts from the fat body invaded the reproductive tissue and the filament of the ovaries, spreading in the epithelial layers and interstitial tissues (Figs. 2, 3, 6).

FIGURE 6. Section through ovary of *A. gambiae*. The microsporidian has destroyed the nutritive cells of the follicles (H), but older better developed follicles are not infected.

Nurse cells were infected. Differentiated follicles were not invaded, so that mature eggs were free of microsporidia. Follicles not yet differentiated at the time of invasion of the ovary were heavily infected, consequently failing to produce mature eggs. This probably reduced the number of eggs produced to half or less of normal. In heavily infected females the nutritive cells were destroyed before the maturation of the follicles and the ovaries were then atrophied. This suggests that artificial distribution of *Nosema* might be useful, in special situations, for control of mosquitoes. The spermatheca and oviducts were not infected in any individual examined.

Morphologic characteristics of the parasite

The bulk of the parasitic forms observed were spores and only a few vegetative forms were seen, but in specific organs small young ovoid schizonts were found.

These were 3–4 microns long and contained a central nucleus. Binuclear stages also were seen, as were the mononuclear merozoites resulting from them. Other stages of schizogony were not found. The sporogonous merozoites, which showed a strong affinity for Giemsa's stain, lost their affinity for the stain after being enclosed within resistant membranes (true cysts). Mature spores were oval with rounded poles and contained a vacuole at one pole. Within these spores a part of the filament and the sporoplasm stained well. In sectioned preparations all normal spores were 2–3 microns long and 1.5–2 microns wide. In some parts of the body, particularly in the gut epithelium and in the fat bodies of the posterior segments, about 10% of the spores present were ovoid macrospores 3×5 microns in size. All these stages define the parasite as a *Nosema*. Pseudocysts, found in the coelomic lumina, were of 2 kinds: Some were in fact epithelial cells from the midgut, distorted and distended by contained spores; others were lymphocytes containing phagocytosed spores or their cyclic products.

Comparing the present infection in *A. gambiae* with the microsporidian infections of mosquitoes discussed by previous authors, many points of close similarity are to be found, along with a few differences. These differences are: (1) The present infection was found in a new host species. In times past this would have been taken as a primary diagnostic feature. We do not give it much weight, believing that a parasitic species may be found in a variety of host species more or less related either systematically or ecologically. (2) The *Nosema* under present consideration does not infect the coelomic lumina nor the air sacs, whereas previously discussed forms were said to have been found in these sites. However, the observations of Marchoux and coworkers (1903) are confused on this point and their record probably is not accurate in this respect. (3) Previous students did not record infection of the Malpighian tubules, whereas the present infection usually is strongly seated in them. We believe that this difference may depend upon the degree of the infection and perhaps upon the different host species. Similarly, infection of the fatty tissue may vary with the host species. On the other hand, growth of the microsporidian in the fat body, leading to massive ejection of spores into the coelom could easily have been mistaken for infection of the coelom itself. When this stage is reached it is difficult to determine the origin of the bodies found in the coelomic lumen and to distinguish true cysts from pseudocysts. (4) Some of the earlier records included spore forms not found in the present infection. It should be noted that in the illustrations presented by Marchoux and coworkers (1903) pseudocysts were identified as true cysts and lymphocytes were identified as plasmodial stages, although nuclei were not seen in them. (5) Minor differences in spore size are not so fundamental; with variation in the host species, some variation in the spores may be expected. The spores of the present *Nosema* measured in sectional material were $2–3 \times 1.5–2$ microns, and in fresh material these same spores before shrinkage were $2.5–4.5 \times 1.7–3$ microns. Similarly the macrospores measured in sectioned material were 3×5, but in fresh material were about 4×6 microns. These measurements fall within the range of measurements recorded for synonymous species.

Accordingly, all the microsporidian infections found in adult *A. gambiae* in the vicinity of Harbel, Liberia, are *Nosema stegomyiae* (Marchoux, Salimbeni and Simond, 1903). Previous records of this parasite have been from *Aedes* (*Stegomyia*) *aegypti*, which has nearly a holotropic distribution, and this may be the basic host species. Because *A. aegypti* shares its ecologic niche with certain anophelines, *A.*

gambiae in coastal Africa, *A. stephensi* in India, and in general with "domestic" anophelines along all tropical coasts, the *Nosema* could have been passed from *A. aegypti* to these ecologically related anopnelines. Differences in detail of the various infections recorded from geographically remote foci evidently were based upon differences in host species and in the severity of the infection, as well as on variation in the evaluation of otherwise probably identical observations by the several students.

Effect of nosematosis on Plasmodium *infection in the anopheline*

During several experimental feedings made with *A. gambiae* on patients with gametocytes of *P. falciparum* it was observed that no oöcysts developed on midguts of mosquitoes heavily infected with *Nosema*, but that where the infection was light or scanty, oöcysts were present but usually covered by clusters of *Nosema* spores which had to be washed away to enable observation; where *Nosema* infection was absent, the *Plasmodium* oöcysts underwent normal development. In 13 lots of *A. gambiae* fed on patients with *P. falciparum* gametocytes, 171 mosquitoes were dissected. Of these, 77 were infected with *Nosema* and in only 3 were oöcysts found. The other 94 mosquitoes from the same lots were free of nosematosis, and 43 of them (46%) had *Plasmodium* oöcysts.

There is no evidence that *Nosema* is physiologically antagonistic to *Plasmodium*, or that the former attacks the latter. In heavily infected mosquitoes the midgut wall is so disintegrated that *Plasmodium* oökinites could not possibly find suitable lodging in the tissue. This would seem to be the basis for the observation that the malaria parasite does not develop in the midgut of the mosquito infected with nosematosis.

Only once was an *A. gambiae* found to harbor both *Nosema* and *Plasmodium* in the salivary glands. In this case the sporozoites of *P. falciparum* were alive but only about half a dozen were found in both glands. The microsporidian had invaded the glandular tissue so extensively that it was "rotten" and dissection was difficult.

Nosematosis in mosquito colonies

Dissection of wild mosquitoes had shown that *N. stegomyiae* is endemic locally. Thus it seems probable that the parasite was introduced to the colonies either with one of the new strains established from wild mosquitoes during the summer of 1956, or from a stray local mosquito that found its way into the insectary rooms.

Study of sectioned material showed that when the ovaries were eventually invaded by the parasite, the eggs already mature were not affected, while the remaining follicles failed to develop further. No parasitic form was found within a mature egg. True transovarian transmission could be ruled out. At most, spores might cling to the exterior of some of the eggs and this could lead to infection of the larvae. No infected larvae were found. Apparently, nosematosis is transmitted from adult to adult mosquito.

Since all infected mosquitoes examined in section had the parasite in the mouth region, and in a few instances only in this region, it seemed clear that the parasite had been ingested. Since both male and female mosquitoes became diseased, the source of contamination must have been something fed upon by both sexes.

It was customary to place into each cage of adult mosquitoes a Syracuse dish containing a small wad of cotton soaked in sugar solution to provide food for the males and supplemental moisture for the females. These cotton wads always had

been discarded after 1 use and there had been no nosematosis in the colony. During the summer of 1956 the supply of cotton ran low and the wads were economically reused, not necessarily in the same cage. The sugar water and the cotton wads could easily have been contaminated with spores either from salivary regurgation or from the feces of infected individuals. Repeated observations showed that just after eclosion the mosquitoes in a cage were free of nosematosis, but dissections made at weekly intervals thereafter revealed succssively higher percentages of infection.

To effect transmission from cage to cage, the reuse of the cotton wads, of the Syracuse dishes, of the cages themselves, would be sufficient. Furthermore, spores could be carried from cage to cage by the sources of blood meals. When animal blood was used to feed the mosquitoes, the animals often were moved directly from cage to cage. Similarly, when the laboratory assistants gave the mosquitoes human blood, they commonly fed the mosquitoes in a series of cages in succession, using the arms and hands.

The very low rate of infection found in the wild mosquitoes is consistent with this view of the method of transmission. Were transovarian transmission usual, the rate of infection in wild mosquitoes should have been much higher. Natural transmission in the wild population probably occurs when the female anopheline takes up water soon after eclosion. That this drinking is usual is well supported by observations of the behavior of the colonized mosquitoes and by the fact that free-living fresh-water organisms were not infrequently found in the midguts of wild mosquitoes which had not yet taken a blood meal when caught.

The question of transmission is not proved experimentally, of course, but our observations strongly support the probability of oral transmission from adult to adult. Those responsible for the management of mosquito colonies might be well advised to be guided accordingly and to avoid any possibility of contamination of glassware, cotton, sugar water, cages, animals or humans used for blood meals.

In the hope that nosematosis could be controlled in the colonies, experiments were conducted with fumagillin, an antibiotic developed by the Abbott Laboratories,* Chicago, Illinois, U.S.A., for use against nosematosis in honey bees. Double the fumagillin activity recommended (Farrar, 1954; Cmejla, 1955; Jamieson, 1955; Cmejla and Otto, 1955; Cmejla, 1956) for use against the bee pathogen, *Nosema apis,* was put into the sugar water and fed to caged adult mosquitoes. At the beginning of the experiments the cages used showed 100% infection with *N. stegomyiae* in both sexes. Eight days later no change in the percentage of mosquitoes infected was noted. Thirty-one days after the beginning of the treatment, the treated cages showed only 50% infection of surviving mosquitoes of both sexes, but the untreated control cages continued to show 100% infection.

Apparently fumagillin acts against *N. stegomyiae* but it is too slow to be of use in mosquito colonies. The average life span of the mosquitoes in the colonies at the Liberian Institute is about 27 days (Fox, 1957). This fact, along with the fact that a mosquito does not take up sugar water on a regular daily basis as does the honey bee, means that by the time Fumidil B is able to produce results, more than half of the mosquitoes in a cage already have died, and the life expectancy of those remaining is too short to make them of any value for experimental work with malaria.

The best hope for control probably lies in prophylaxis, including prompt destruction of infected batches of mosquitoes.

* Fumidil B, Abbott.

SUMMARY

Nosema stegomyiae infections of *A. gambiae* and *A. melas* from Liberia were studied both in fresh preparations and from stained serial sections of infected mosquitoes. Diseased mosquitoes cannot be recognized from external characteristics but can be diagnosed from dissection of the midgut. Sectioned material shows that the microsporidian invades all organs and tissues except the air sacs, tracheae and coelomic lumina. Parasites enter at the mouth; esophageal and salivary gland tissues are infected at the outset and the infection spreads long the alimentary tract, including the malpighian tubules, and then to fatty tissues, muscular tissues, ganglia, and circulatory epithelium and phagocytes. In undifferentiated ovarian follicles the epithelial, interstitial and nurse tissues are involved, leading to follicular atrophy and a curtailing of the number of eggs produced.

Differences in morphology and pathology between this infection and those recorded by other authors are noted. These are slight and probably depend upon differences in the host species concerned.

Transmission is probably oral through ingesting contaminated food; transovarian transmission does not seem to occur; no infected larvae were found.

In *A. gambiae* having nosematosis, *P. falciparum* oöcysts develop only where the *Nosema* infection is light because the midgut epithelium disintegrates after invasion of the parasite and thus denies suitable lodging to the *Plasmodium* oökinetes.

Fumidil B, an antibiotic developed by Abbott Laboratories for use against nosematosis in honey bees, also is effective against *N. stegomyiae* in *A. gambiae* but its action is too slow to be useful in treating infected mosquito colonies. The best control is prophylactic sterilization of all cages and equipment.

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EXPERIMENTAL TOXOPLASMOSIS IN CHICKENS

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The chicken is a natural host of *Toxoplasma gondii*. The parasite has been reported from birds with paralytic or spastic symptoms (Hepding, 1939; Fankhauser, 1951) and has also appeared in epizootics among flocks in Norway (Ericksen and Harboe, 1953). Experimental infections in chickens have been studied by Levaditi and Schoen (1928) who found only young chicks susceptible, by Jacobs and Jones (1950) who studied parasitemias, and by Harboe and Erickson (1954). The latter authors were not able to duplicate, in their experimental birds, the symptoms found in the flock in which they discovered epidemic toxoplasmosis in spite of the fact that they used parasites isolated from a sick bird. According to Siim (1956) Dr. Biering-Sørensen has found an acute spontaneous infection in hens in Denmark and has demonstrated toxoplasmas in the ovaries and in eggs of some birds. Gibson and Eyles (1957) reported finding infected chickens in an area neighboring the home of a case of human toxoplasmosis in Memphis, Tennessee.

Especially in view of the widespread distribution of *Toxoplasma*, it is necessary to evaluate the potentialities for its spread among individuals of each host species and from one species of host to another. The importance of the demonstration of parasites in chickens and in hens' eggs requires evaluation as to how frequently such localization of *Toxoplasma* occurs. In addition, the flesh of the chicken, as a food for man, requires study as to the frequency with which it harbors toxoplasmas. Furthermore, the parasitemia is of interest in regard to the possibility of arthropod transmission. In addition to these motives for further investigation, base line studies on the chicken were desirable to see if it would be useful in chemotherapeutic trials. Incidental to such work, dye test titers have been obtained on chickens with experimental infections, with the view to evaluating the usefulness of serological tests in surveys of flocks.

The studies to be reported here were performed in 2 Sections of this Laboratory. The results are combined to give a more complete picture of the findings.

MATERIALS AND METHODS

Chickens of the Hampshire Red and the White Wyandotte breeds were used. The age of the birds will be stated below in connection with the individual experiments. The chickens were infected with toxoplasmas of the RH, 113-CE, and NR-139 strains by a variety of routes, using conventional techniques. The inocula were prepared from peritoneal exudates of infected mice or membranes of infected chick embryos by counting the parasites in a Neubauer-Levy chamber and adjusting the suspensions by dilution so that the desired number of organisms was obtained. In some experiments relatively small inocula, such as 300 to 10,000 parasites, were given. In

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others, very large inocula of 10 or 100 million parasites per kilogram of weight were used in attempts to reproduce the clinical disease found by others.

For studies of parasitemia, whole blood withdrawn by cardiac puncture was heparinized and inoculated intraperitoneally into mice. Each mouse received 0.2 ml. For studies of the distribution of parasites in the tissues, pieces of tissue were weighed and a volume of saline 10× this weight was added. The material was then ground in a mortar with sand, and 0.5-ml aliquots of the resulting suspension were inoculated intraperitoneally into each of 2 mice.

The time to death of the mice was noted. In the case of survival, the mice were killed, and suspensions of their brain, liver, and spleen were subinoculated into 2 fresh mice. Usually only 1 such subinoculation was performed.

Clinical symptoms were noted by daily observation of the chickens in some of the studies. Temperatures were recorded in some cases. Malaise was judged by general appearance and by such specific points as stance and response to stimuli, and especially by the paleness and flaccidity of the comb. Weakness was judged by gait and the inability of the chicken to rise when down. Loss of weight and diarrhea were easily noted. Encephalitic symptoms included either retraction of the head or jerking movements.

RESULTS

Parasitemia and clinical features

The level of infection found in the blood of chickens was studied in birds inoculated with varied numbers of RH toxoplasmas by different routes. The data of these tests are summarized in Table I.

TABLE I — *Parasitemia in chickens infected with* Toxoplasma gondii

No. of birds	Weeks of age	Inoculum Size	Route	Parasitemia*	Remarks
1	6	5000 RH	IV	4, 7	Survived
1	6	5000 RH	IV	8, 11	"
2	6	5000 RH	IV	4, 8, 11, 15, 22	"
3	1	300 RH	IV	3, 6, 7, 10, 12, 14, 17, 19, 21, 23†, 26†	"
4	10-15	1×10^6/kg RH	IC	2	Died days 3 & 4
2	10-15	1×10^6/kg RH	IC	2, 5	" " 5 & 7
1	10-15	1×10^5/kg RH	IC	2, 5	Survived
1	10-15	1×10^5/kg RH	IC	2, 5, 8	"
4	10-15	1×10^5/kg RH	IC	2, 5, 8, 12, 15, 19	"
1	10-15	1×10^8/kg RH	SC	2, 4, 7	Killed day 7
3	10-15	1×10^8/kg RH	SC	2, 4, 7, 10, 14, 17, 21, 24, 28†, 31†	
3	10-15	1×10^7/kg RH	SC	2, 4, 7, 10, 14, 17, 21, 24†, 28‡, 31‡, 35‡, 46‡	
3	laying hens	1×10^8/kg RH	IV	3, 5‡, 7	3 died
3	laying hens	1×10^8/kg RH	IV	3, 5, 7, 9, 11, 13, 15, 17‡, 21†, 29†	
6	laying hens	1×10^7/kg RH	IV	3, 5, 7, 9, 11, 13, 15, 17▲	
9	10-15	1×10^8/kg RH	IV	2, 3 5, 7, 9, 10, 11, 13, 15‡, 16‡, 17‡, 18†, 19♦, 20-34‡	
9	10-15	1×10^7/kg RH	IV	2§, 4, 7, 9, 13, 16, 19-20, 22-23, 25-27, 29‡, 34§	

*Figures given are days of infection on which parasitemia was tested; day of infection = day 0. Underlined figures indicate days parasites were found in the blood. Number of lines under a figure indicate number of birds found positive that day.

†Only 1 bird tested ‡Only 2 birds tested. §3 birds tested. ♦4 birds tested.
▲All tests positive on all birds tested.

First, it is to be noted that the only chickens that succumbed to toxoplasmosis were those given inoculations of 1×10^6 or more parasites. Intracerebrally infected birds all died with symptoms of severe encephalitis within 1 week, only 2 surviving

beyond day 4 after inoculation with 1×10^6 toxoplasmas per kilogram. On the other hand, 4 of 6 chickens of the same age, 10 to 15 weeks, and weighing at least 1 kg survived for over 2 weeks following intracerebral inoculation of 100,000 toxoplasmas per kg. Of the other 2, 1 was moribund and was killed on day 5, and the other was killed on day 10. These chickens showed no parasitemia beyond day 8 of infection.

Subcutaneous inoculations of 10- to 15-week-old chickens produced only a mild febrile episode despite the injection of enormous numbers of parasites. No symptoms were noted, but temperature readings showed about a 2-degree rise on the 7th or 8th day, persisting at least through day 10. All of the birds had parasitemia during the first week, and 2 of them were found still to have parasites in the blood as late as the 4th or 5th week after infection.

Following intravenous inoculation, chickens 10 to 15 weeks old which received 10^5 RH parasites per kg had parasitemia throughout the first week of infection, and some of the birds continued to show parasites in the blood for 2 weeks, although in diminished numbers as revealed by longer survival time of mice inoculated with the blood. All of the birds showed a temperature rise of 1 to 3.5 degrees F. One bird showed encephalitic symptoms, manifested by jerking head movements; the others manifested no symptoms other than the fever and an attitude of malaise during the first few days. Chickens of the same age, infected with 10^7 RH parasites per kg showed parasitemia in practically all those tested on days 2 and 4, and occasional positive bloods were found in individual birds thereafter, on tests done up to day 27. Only 1 of these 9 birds showed a fever, a 2.5° F rise in temperature; all the others appeared unaffected by the infection except for early malaise up to day 3. After day 7, some of these chickens developed a severe diarrhea, very pale combs, and general weakness. This was suspected as due to an unrelated infection, but no proof for this was found. Seven of 12 laying hens inoculated intravenously with 1×10^7 or 1×10^8 parasites also had prolonged parasitemia, persisting slightly beyond 2 weeks. Three of the hens, which received 1×10^8 toxoplasmas per kg, succumbed to the infection.

Other chickens, under 1 week of age and weighing between 40 and 60 grams, received small numbers of parasites intravenously. These showed parasitemias similar to those previously reported (Jacobs and Jones, 1950). Another group of older birds, aged 6 weeks, received inocula of 5,000 RH toxoplasmas intravenously. There was an occasional low-grade parasitemia in 3 of the 4 birds during the first week, as judged by the prolonged survival time of mice inoculated with their blood, but these chickens all survived without evidencing any symptoms.

Serology

Results of dye tests on a large number of the older chickens are presented in Table II. It is to be seen that there was a rapid response, with cytoplasm-modifying antibody demonstrable by day 7 in most of the cases. It is also remarkable that dye test titers on these birds did not exceed 1 : 64 and rapidly declined to lower levels. Birds given inocula of millions of parasites showed titers as low as 1 : 4 five weeks after infection. Parasitemia persisted in some birds after the appearance of antibodies. This may be attributable to the low titer of antibodies and possibly to the occurrence of intracellular parasites in the blood. The intracellular position protects parasites from the action of cytoplasm-modifying antibody, according to Sabin and Feldman (1948).

TABLE II — *Dye test results on chickens infected with* Toxoplasma

Bird number	Age	Size of inoculum	Reciprocal of titer on days							
			4-5	7	9-11	13-15	19-22	27-36	37-44	50-72
C – 601	laying hen	10^8/kg				64	64		64	
603	"	"		16		64	16	neg.	4	
604	"	"		4						
607	"	"			16	64	16		16	
606	"	10^7/kg	neg.	64		64				
608	"	"		64		64		4		
609	"	"	neg.	64		64		16		
610	"	"		4		16	16		4	
611	"	"		64	64					
612	"	"		4		16			4	
613	10-15 weeks	10^8/kg		1		64		16		
615	"	"		64		64				
616	"	"		64		64	64			
617	"	"		64		64	64	16	16	
618	"	"		64						
619	"	"		64						
620	"	"		64		64	64	16	1	4
621	"	"		64		16	16	4	1	0-4
622	"	10^7/kg		64		16	16	16	0	0-1
623	"	"		16		64	64	64	1	0-4
624	"	"		64		64	16	4	1	0-4
625	"	"		16	64	16	4	4	4	4
626	"	"		16	64	64	16	16	4	16
627	"	"		4	64	16	16	16	4	
628	"	"		64	16	64	16	16	4	
629	"	"		64	16	64	16	4	4	
630	"	"		64	64	64	4	16		

Distribution of parasites in tissues

Because of the generalized distribution of parasites early in the infection, the possibility was considered that despite their tolerance, chickens might nevertheless harbor *Toxoplasma* in latent form for long periods, as do pigeons (Jacobs, Melton and Jones, 1952) rats (Ruchman and Fowler, 1951) and other hosts. A number of tests was made of the persistence of *Toxoplasma* in the tissues of infected chickens. In the case of the older birds, the tests were confined to the brain, liver, and lung. In younger birds, more organs were studied.

Autopsy results on 10 to 15-week-old chickens infected with millions of parasites by intracerebral or subcutaneous routes are shown in Table III. Birds which died or were killed during the first or second week after infection showed toxoplasmas in the brain, liver, and lung. One bird killed during the third week after infection had parasites in the brain but none in the lung or liver. Of 8 birds killed 3 or more weeks after infection, only 1 was found still to harbor parasites and in this case they were found only in the liver.

The 10- to 15-week-old birds that were infected intravenously with 100 million toxoplasmas per kg showed similar results. Of 5 chickens autopsied 26 or more

TABLE III — *Demonstration of parasites in the tissues of chickens infected intracerebrally or subcutaneously with* Toxoplasma

Bird number	No. of parasites/kg inoculated	Route	Died, killed, or sacrificed †	Autopsy day*	Method of demonstration ‡	Presence of parasites		
						Brain	Liver	Lung
C – 638	10^6	IC	D	4	S	+	–	–
C – 639	10^6	IC	D	4	S	+	–	–
C – 640	10^6	IC	D	3	I	+	+	+
C – 644	10^6	IC	D	4	S	+	–	–
C – 645	10^6	IC	D	5	S	+	–	–
C – 646	10^6	IC	D	7	I	+	+	+
C – 641	10^5	IC	K	5	S	+	–	–
C – 642	10^5	IC	S	30	I	–	–	–
C – 643	10^5	IC	K	19	I	+	–	–
C – 647	10^5	IC	S	23	I	–	–	–
C – 648	10^5	IC	K	12	I	+	+	+
C – 649	10^5	IC	K	16	I	+	–	–
C – 631	10^8	SC	S	28	I	–	–	–
C – 632	10^8	SC	K	7	I	+	+	+
C – 633	10^8	SC	S	32	I	–	–	–
C – 637	10^7	SC	K	27	I	–	+	–
C – 634	10^7	SC	S	24	I	–	–	–
C – 635	10^7	SC	S	32	I	–	–	–
C – 636	10^7	SC	S	53	I	–	–	–

*Day of infection = day 0. †K = accidentally killed ‡S = smear; I = mouse inoculation

days after infection, one had parasites in the brain. Chickens infected intravenously with 10 million parasites were negative for parasites in the brain and liver when killed on days 36 to 72.

Laying hens infected intravenously had a prolonged parasitemia, as already noted. Consistent with this finding, a larger number of these chickens was found to have parasites when autopsied late after infection. Of 8 hens killed on days 33 to 41, 4 had toxoplasmas in the brain, and 3 of these also showed parasites in the liver; and an additional hen showed parasites only in the liver. These findings suggest that the laying hen is not as capable as other chickens of ridding herself of toxoplasmas; possibly this is due to hormonal influences, or to nutritional requirements which were not completely satisfied on the laboratory diet. However, even in these birds, the persistence of parasites in the tissues was not a uniform occurrence, and it must be remembered that they were inoculated with enormous numbers of parasites, 1×10^8 toxoplasmas per kg.

Some tests also were made of the persistence of *Toxoplasma* in muscle. For this purpose, 6-week-old birds were infected with 5000 parasites intravenously. These chickens were killed at weekly intervals and their tissues were inoculated into mice. Results of these tests showed that there was a rapid disappearance of toxoplasmas from the tissues. At 1 week, the liver, spleen, lung, heart, kidney, and bone marrow were infective to mice; brain, breast muscle, and leg muscle were negative. The sam organs were tested in birds killed 2, 3, and 4 weeks after inoculation. At 2 weeks only spleen and heart, and at 3 weeks only heart and breast muscle were positive. At 4 weeks, all these organs were negative. Even after 2 and 3 weeks, loss

of parasites was evident not only in the fewer tissues found infective, but in longer survival time of mice inoculated with them.

Similar tests also were performed with 8-day-old chickens, to see if the younger birds would be equally as capable of clearing their tissues of parasites. The results were no different from the earlier tests; parasites were recovered only rarely 4 weeks or longer after infection.

In the same series as the last-described test, a group of 8-day-old chicks was infected with toxoplasmas of the RH strain which had been maintained continuously in chick embryos for several years. This chick-embryo-passed line was not strikingly different from the mouse-passaged line in the distribution or survival of toxoplasmas in the tissues.

Attempts to modify susceptibility

Because of the ability of chickens to tolerate massive infection with *Toxoplasma* in the laboratory, as contrasted with the severe epidemic disease in flocks studied in Norway, it was considered possible that the susceptibility of birds could be varied under some circumstances. To test the possibility that diet could influence the course of chicken toxoplasmosis, 2 tests were made of dietary deficiencies.

Four-week-old chickens which had been maintained on standardized diets since hatching were obtained from the Laboratory of Nutrition and Endocrinology, National Institute of Arthritis and Metabolic Diseases, through the courtesy of Dr. M. R. Spivey Fox. The diets were C8 and C17, both corn-soy regimens with 4% and 24% fat respectively. Test groups had the C17 diet lacking in vitamin B12. These chickens were inoculated with 10,000 RH toxoplasmas subcutaneously. Again, the results showed no differences between the vitamin-deficient groups and the groups receiving the complete diets as to their susceptibility to *Toxoplasma* and as to the distribution of parasites in their tissues. Similar results were obtained with chicks on a folic-deficient diet, C12, obtained from the same source.

In another attempt to modify susceptibility, 4-week-old chicks were given cortisone subcutaneously at dosages of 5 or 10 mg per day for 2 days prior to intravenous inoculation with 10,000 RH toxoplasmas, and then for an additional 2 days. No symptoms were observed in these birds, and they all survived as well as the controls.

DISCUSSION

The tests reported above indicate that the chicken is remarkably tolerant to toxoplasmosis. Only large inocula are capable of producing disease in mature fowl and even very young chickens are able to survive infections produced by inoculation of numbers of the same strain of *Toxoplasma* that are generally fatal to laboratory animals, such as rabbits, guinea pigs and hamsters.

Parasitemia occurred in young birds as early as 3 days after inoculation with relatively small numbers of parasites, and after 2 days in larger fowl inoculated with greater numbers. The parasitemia was not constant or progressive in either case. Despite the size of the inoculum it only occasionally persisted more than 2 weeks after inoculation. Thus, despite its resistance, the chicken does experience an acute stage of toxoplasmosis, with proliferation and dispersal of organisms throughout the body.

On the other hand, after this dispersal of parasites, the chicken also develops a remarkable ability to clear itself completely of the infection. Despite the enormous

numbers of parasites given to large birds, and the persistent parasitemias in some cases, toxoplasmas were rarely found in the tissues later than day 40 after inoculation. It is also noteworthy that while the chick embryo succumbs to relatively small inocula of toxoplasmas of a strain of low virulence (Jacobs and Melton, 1954) even very young chicks survive inoculation with RH parasites and have the ability rapidly to clear their tissues of the infection.

The results of our attempts, by modification of diets and by use of cortisone, to render them less resistant to toxoplasmosis, do not indicate that such factors can be responsible for the difference between the epidemic form of the disease in the chickens and the laboratory-induced infection. Obviously, our tests were merely exploratory and more work will be required to rule out factors of this type. It would seem worthwhile, however, to investigate the possibility that the epidemics were due to a concomitant infection as well as toxoplasmosis.

Our data also negate the hypothesis that chicken flesh itself could serve as a source of infection for humans. Chickens apparently clear their muscles of parasites very rapidly. Also, in view of the rapid clearing of the tissues in laying hens which received very heavy inocula, it seems unlikely that the presence of *Toxoplasma* in hen's eggs is anything other than an occasional finding.

It is obvious, from the serological test results on these chickens, that the dye test reveals only very low levels of antibody in infected fowl. Any positive dye test reactions found in chickens, in survey work, are therefore interpretable as representing the presence of active or very recently active infection.

The chicken obviously lacks value as an experimental host for chemotherapeutic studies on toxoplasmosis. What is intriguing about this host is its low level of dye test antibody in the presence of a strong immune reaction as evidenced by its ability to clear its tissues of parasites. From this standpoint the chicken offers, possibly, an opportunity to study the relative importance, in immunity, of cytoplasm-modifying and other antibodies versus other humoral factors and cellular factors.

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THE LETHAL EFFECT OF *EIMERIA TENELLA* EXTRACTS ON RABBITS*

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Recent observations on host-parasite relations in cecal coccidiosis indicate that this disease is manifested by diverse histo-pathological and early physiological changes in the chicken (Bertke, 1955; Levine and Herrick, 1954; Schildt and Herrick, 1955). Bertke suggested that the changes seen in organs far from the site of the primary infection might be due to a toxic substance released by or through the action of the parasite. A search for toxic substances released during *Eimeria tenella* infections was undertaken in conjunction with studies on immunity in this disease. One approach in the investigation was an attempt to induce the formation of coccidial antibodies in rabbits by injecting them with extracts prepared from cecal contents of infected chickens. The injection of relatively small amounts of this material intravenously or intraperitoneally into laboratory rabbits resulted in the death of the animals in 16 to 24 hours. This is a report on initial studies of extracts from *Eimeria tenella* and their toxic effect on rabbits. The possible significance of this toxin in the coccidiosis syndrome is discussed.

MATERIALS AND METHODS

The chickens used in these experiments were Single Comb White Leghorns supplied by the Department of Poultry Husbandry of the University of Wisconsin. The birds were 1 day old when delivered and were reared in a separate building until needed for experimental purposes. All feasible precautions to keep the birds free of coccidia were employed. The birds were 8 to 12 weeks old when experimentally infected.

The oocysts used for infection were from a pure strain of *E. tenella* serially transferred for many years in this laboratory. They were allowed to sporulate in 2.5% potassium dichromate. Heavy infections were obtained in chickens given 150,000 sporulated oocycts orally.

Toxin was obtained from 2 sources: The cecal mucosa of infected chickens, and isolated oocysts. The lethal effect in rabbits was first noted following injections of extracts prepared from the ceca of infected chickens. Heavily infected ceca were obtained on the 5th day of infection. The contents of the ceca and scrapings from the mucosa were placed in 20-ml capacity glass homogenizers. Saline was added in amounts which allowed for effective homogenization without excessive dilution of the cecal material. The mixture was homogenized and centrifuged. The supernatant fluid obtained was referred to as crude cecal extract and was lyophilized or kept in the refrigerator as a liquid. Extracts from infected and normal ceca were prepared in the same fashion except that the contents of the normal and the 1st- to 4th-day infected ceca were discarded before the walls were scraped to obtain mucosa for homogenization. This was done to avoid excessive contamination with the fecal material which is found in non-hemorrhagic ceca.

Partial purification of crude extract was effected by precipitation with saturated ammonium sulfate. Lyophilized crude extract was resuspended in distilled water (10 mg/ml). This was diluted by half with saline. Saturated ammonium sulfate was added dropwise from a burette at room temperature (techniques modified from Becker et al, 1951). The solution was constantly agitated as the ammonium sulfate was added. The mixture was centrifuged when it contained

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20% ammonium sulfate. The precipitate obtained was resuspended in an equal volume of saline and the supernatant (considered to be 20% saturated ammonium sulfate) was treated with additional salt. In this way the cecal extract was precipitated in fractions, each fraction dissolved in an equal volume of saline and each tested for toxic activity. Toxic substances were found to be present in precipitates from 40% to 55% saturated ammonium sulfate. Sediment from extracts precipitated in this range was resuspended in saline and dialyzed to remove the ammonium sulfate. The dialyzed preparation was lyophilized or used in further attempts to purify the extract.

Further purification was effected through adsorption with tricalcium phosphate [$Ca_3(PO_4)_2$]. An adsorption column was prepared by packing a glass tube 12 inches in length and 1 inch in diameter with granular tricalcium phosphate and equilibrating with a 0.1N phosphate buffer solution (pH 7.4). A 5.0-ml sample of ammonium sulfate-precipitated extract was added to the top of the column. As the extract moved down the column, buffer solution was added dropwise to keep the tube saturated. A red band (hemoglobin?) from material present in the extract was observed to move through the column. Collection of fractions was discontinued when this band reached the bottom of the tube (18 to 24 hours). The toxic material had passed through at that time. The filtrate was then dialyzed and the dialyzate lyophilized.

Although extracts from infected cecal pouches contained a toxic substance, it was not known whether the toxin originated from host tissue modified through the action of the parasite or from the parasite itself. The possibility that the toxin was wholly parasitic in origin was investigated by preparing extracts from sporulated *E. tenella* oocysts. The total number of oocysts used in preparing an extract was never under 50 million and averaged 250 million. These were concentrated by flotation techniques using saturated sodium chloride. The oocysts so obtained were washed by centrifugation in distilled water. Washed oocysts were placed in a pepsin digestion medium (7.5 mg commercial pepsin/1.0 ml 0.1N HCl) and stirred constantly at 37° C for 4 hours. Centrifugation of this mixture resulted in a brown layer of extraneous material overlying a white layer consisting almost entirely of oocysts. The brown layer was scraped off and the oocysts washed by repeated centrifugations. Finally the oocysts were suspended in a small amount of saline and macerated at 10° C in a glass homogenizer until few intact oocysts remained in the suspension. The homogenate was centrifuged, the supernatant collected and stored in the refrigerator for future use. This preparation was referred to as oocyst extract and its strength expressed in terms of the number of oocysts homogenized per ml of extract obtained (generally 15,000,000/ml).

RESULTS

Effect of extracts on rabbits. The injection of crude cecal extract intravenously or intraperitoneally into rabbits resulted in the death of the animals in 16 to 24 hours (Table I). It was difficult to standardize the amount of extract that constituted a

TABLE I. *Effect on rabbits of injections of* Eimeria tenella *extracts.*

Nature of extract	Route of injection and dosage		Number of rabbits tested	Effect*
	IP	IV		
Crude cecal extract from normal chickens	5.0 ml	3.0 ml	12	0
Crude cecal extract from chickens infected for				
1–2 days	4.0 ml	2.0 ml	8	0
3 days	2.0 ml	1.0 ml	8	±
4 days	2.0 ml	1.0 ml	10	+
5 days	2.0 ml	0.5 ml	12	+
6 days	2.0 ml	1.0 ml	9	+
7 days	2.0 ml	1.0 ml	7	±
8 days	2.0 ml	1.0 ml	8	0
Lyophilized infected cecal extract		5–10 mg	9	+
$(NH_4)_2SO_4$-precipitated infected cecal extract		5–10 mg	7	+
$Ca_3(PO_4)_2$-adsorbed infected cecal extract		5–10 mg	5	+
Oocyst extract before rupture		1.0 ml of 5×10^7/ml	6	0
Oocyst extract after rupture		1.0 ml of $2–4 \times 10^6$/ml	12	+

* Effect was graded as follows: 0 = none; ± = mildly toxic; + = lethal.

minimal lethal dose when dealing with the crude preparation. Major factors, including the severity of the infection in the cecum from which the extract was prepared and the amount of saline with which the extract was diluted for homogenization, influenced the potency of the toxic preparation. However, it was shown

that very little of the extract was necessary to cause death in the rabbit. In one experiment, 7 rabbits were given 0.05 ml of the crude extract intravenously. Within 24 hours, 4 were dead and the remainder obviously distressed. Five days later the 3 remaining animals, apparently recovered, were given 0.10 ml of the same material. All 3 were dead within 24 hours.

Extracts prepared from normal cecal mucosa had no toxic effect on rabbits. Preparations from ceca taken from chickens after 1 and 2 days of infection were also non-toxic. The injection of 1.0 ml cecal extract from chickens infected for 3 days produced mild symptoms in the rabbit. The animal became listless, refused to eat, and in some cases showed mild diarrhea. During the 4th, 5th, 6th and 7th day of infection, however, the cecal material was toxic, with the most potent action noted in that prepared during the 5th day. Beyond the 7th day of infection the toxin could not be recovered from cecal material homogenized in this way (Table I).

Symptoms in rabbits following the administration of a lethal dose of toxic extract were fairly consistent. For a few hours after injection the animals appeared restless. After approximately 5 hours, the rabbits remained quiet, the respiratory rate was accelerated and breathing appeared labored. Twelve hours after injection the animals were usually nearly prostrate, diarrhea was present, the respiratory rate was double the normal and quite irregular. The animals died between 16 and 24 hours after the injection. Death was preceded by extreme respiratory difficulties, convulsive seizures and finally collapse. Convulsions began about 5 minutes before breathing stopped.

The only consistent *post-mortem* finding was an accumulation of fluid and gas in the intestine. The small intestine was in most cases devoid of food. The stomach was filled with food, although this appeared to be the case also in normal rabbits after being off feed for 24 hours. Scattered hemorrhagic areas in the lungs were occasionally observed. The spleen was sometimes dark red and enlarged. Other organs showed no gross pathology. Animals given sub-lethal doses of toxin often showed the same symptoms of listlessness and prostration, with slow recovery over a period of 48 hours. In these, diarrhea was more pronounced and frequently mucus-like discharges from the nose and eyes were apparent. Respiratory difficulties were always present.

The same sequence of symptoms followed when lyophilized crude, ammonium sulfate-precipitated, or calcium phosphate-adsorbed extracts were given (Table I). Little difference was found in the lethal doses of these preparations. The minimal lethal dose was 5 to 10 mg for each extract. Extracts from oocysts affected rabbits in the same way as did toxic cecal preparations. Extract containing from 2 to 4 million macerated oocysts per ml probably represented the minimal lethal dose. Unsporulated oocysts also released toxin when homogenized.

The effect of homogenization of oocysts was shown by the following experiment. A 10.0 ml suspension of 470 million sporulated oocysts was allowed to stand in the refrigerator for 8 days. It was then centrifuged and 1.5 ml supernatant was injected intravenously into a rabbit. No toxic effect was evident. The oocysts were then macerated, the homogenate centrifuged and 0.5 ml supernatant injected into a rabbit. The rabbit died within 16 hours.

Effect of extracts on other animals. All preparations that were shown to be toxic for rabbits caused no obvious toxic reactions in chickens. Indications of a drop in body weight and various other responses to the injections of toxic material

were insignificant when compared to adequate controls. Treatments that could not be used on chickens were the intravenous inoculations of crude cecal extract and lyophilized crude extract. These caused intravascular coagulation due to the thromboplastic effect (Bradford et al, 1947). However, these preparations could be given intraperitoneally in large doses without causing the clotting phenomenon. The other extracts could be given intravenously to chickens without causing intravascular coagulation.

Other laboratory animals also proved to be refractory to the toxic substance. Five ml of crude cecal toxin (0.5 ml lethal to rabbit) given intraperitoneally to rats, guinea pigs and hamsters caused no toxic reactions. One ml of the same material intraperitoneally and 0.1 ml intracerebrally into mice did not elicit toxic symptoms.

Toxin-neutralizing antibodies. Attempts were made to induce the formation of toxin-neutralizing antibodies in rabbits and chickens by injecting them with the various toxic preparations described (Table II). Because of the variety of extracts

TABLE II. *Results of attempts to produce toxin-neutralizing antibodies in rabbits.*

Extract used#	Approx. Lethal dose (ml)	Injection schedule and amount (ml) of extract administered*												No. of rabbits immunized	No. of rabbits producing neutralizing antibodies
		First series								Second series**					
		First injection	Days after first injection 2	3	4	5	6	7	9	1	3	5	7		
Crude	1.0	0.2	0.5		0.5		0.5			0.5	1.0	1.0	2.0	2	1
Crude	0.5	0.1	0.1		0.25		0.3			0.3	0.5	0.5	1.0	3	0
CL	0.5	0.2	0.2		0.3			0.3		0.4	0.4	0.5	1.0	4	2
CL	1.5	0.25	0.5			1.0		1.5	2.0	1.0	1.5	2.0		3	1
CL	0.5	0.2	0.25			0.3		0.5	0.5	0.5	0.5	1.0	1.5	2	0
ASP	1.0	0.1	0.1		0.2		0.2			0.2	0.3	0.3	0.5	3	0
ASP	1.0	0.2	0.2			0.3		0.5	0.5	0.5	0.7	1.0	1.0	3	0
CPA	1.0	0.5	0.5			0.5		0.5		0.5	1.0	1.0	1.0	3	0
CPA	1.0	0.25	0.25		0.3		0.5			0.5	0.5	1.0	1.5	3	0
Oocyst extract 6×10^6/ml	0.5	0.2	0.25		0.25			0.3		0.3	0.4	0.5	0.5	3	0
Oocyst extract 1×10^7/ml	0.5	0.1	0.1		0.2		0.2			0.2	0.3	0.3	0.5	3	1
Oocyst extract 7.5×10^5/ml	0.5	0.25	0.15			0.2		0.25	0.4	0.3	0.5	1.0	1.0	3	0
Oocyst extract 2×10^7/ml	0.3	0.1		0.1		0.2		0.25		0.3	0.3	0.4	0.5	3	0

* All injections via marginal ear vein; all animals bled one week after the last injection.
** Initiated one week after the last injection.

\# Crude = Crude infected cecal extract
CL = Crude lyophilized extract (10 mg/ml)
ASP = Ammonium sulfate-precipitated extract (10 mg/ml)
CPA = Calcium phosphate-adsorbed extract (10 mg/ml)

used and their effects on rabbits, no rigid dose or schedule of injections could be established for that animal. As a rule, rabbits were given 4 sublethal injections of a toxic extract over a period of one week and another series of 4 injections of slightly larger amounts a week later. Injections were made in the marginal ear vein. Chickens (8 to 12 weeks old) were given 2 or 3 intraperitoneal or intravenous (via wing vein) injections of toxic extract on alternate days. The amount of extract given in one injection varied from 0.5 ml to 4.0 ml; the total amount given any one bird ranged from 1.5 ml to 7.0 ml. Blood was collected from rabbits and chickens 1 week after the last injection by cardiac puncture. The collected blood was allowed to stand overnight in the refrigerator and the serum separated from the clot by centrifugation on the following day.

Serum was also obtained from rabbits that had received a series of 4 injections of normal cecal mucosal extract on alternate days, the amount of the injection having been increased from 0.5 ml on the first day to 2.0 ml on the last day of the series. Serum from chickens recovered from experimental coccidiosis was also examined for toxin-neutralizing activity.

Samples of each serum obtained by the methods given above were tested for the presence of toxin-neutralizing antibodies. One ml rabbit serum was mixed with 1 to 2 ml of toxic extract in 15-ml centrifuge tubes. The tubes were placed in a 37° C incubator for 1 hour, transferred to the refrigerator and allowed to stand overnight. The mixture was then centrifuged and the supernatant fluid injected into rabbits as a test for toxicity. If the material proved to be non-toxic, the same extract was mixed in the same proportion and treated in like manner with serum from rabbits immunized with normal cecal mucosa extract. This was given the same rabbit 2 days after the test injection and served as a control. Serum was considered positive for neutralizing antibody only if the control injection was lethal. The same procedure was followed using chicken serum except that a wider range of extract-serum ratios was tested and the salt concentration of the mixture was often increased to 8% (Goodman et al, 1951).

Fourteen rabbits received the immunizing series of injections of crude cecal extract and lyophilized crude extract outlined above (Table II). Of them, 4 produced serum that neutralized the toxic extracts. The toxin-neutralizing property of the serum from each of the 4 rabbits was tested separately on 4 different rabbits (a total of 16 rabbits) and found to be present in every case. Up to 10 MLD were neutralized by 1.0 ml of antiserum from these animals. In addition, these antisera were also found to neutralize the toxin in ammonium sulfate-precipitated, calcium phosphate-adsorbed and oocyst extracts.

Rabbits which failed to produce neutralizing antibodies that could be demonstrated *in vitro* nevertheless developed some resistance to the toxic material. Table II shows that by the time the immunizing series of injections was completed, the animals were receiving amounts which equaled or exceeded the lethal dose. It should be pointed out, however, that the rabbits listed in Table II represent only those that survived the entire series of injections. Occasionally an animal died during the immunization procedure, apparently from the effects of the extract.

Neutralizing antiserum without exception strongly precipitated the toxic extracts. Antiserum which proved to be ineffective as far as neutralization was concerned also precipitated toxic extracts, though less regularly. Antiserum against normal mucosa also caused precipitation of infected cecal preparations. This held true for all toxic extracts. Consequently, the use of a precipitation test as an indication of the presence of antitoxin in the serum was considered to be completely unreliable.

Serum that neutralized toxin when the two were mixed, incubated and injected together was also tested for its ability to neutralize toxin when the two were injected separately. Rabbits given a lethal dose of toxin were also given a total of 6.0 ml neutralizing antiserum in 3 equal injections, 3 hours apart, the first given immediately before the toxin was introduced. The animals showed no signs of distress. The same procedure, using anti-normal cecal mucosa serum, served as a control. This serum failed to protect rabbits from the lethal effects of the extracts.

Six rabbits were given a series of immunizing injections of ammonium sulfate-precipitated extract and 6 were given calcium phosphate-adsorbed extract. None

of these animals produced neutralizing antibodies against the homologous toxin or any other toxic extract. Some sera from these precipitated crude cecal and oocyst extracts as well as the homologous extract. Of 12 rabbits given oocyst extract, only 1 responded by producing antibodies capable of neutralizing all toxic extracts (Table II).

Each preparation was administered to chickens in attempts to produce toxin-neutralizing antibodies. The doses were varied and the courses of injections were manipulated to try many combinations. Mixtures of extract and antiserum were made up in ratios of 1 : 0.5 up to 1 : 10. Serum from chickens recovered from 1 and 2 experimental infections of coccidiosis taken at different times after infection and incubated with toxic extracts in the variety of ways listed above were tested. All of these tests showed that chicken serum, no matter how produced or how treated, was not able to neutralize any of the toxic preparations. Although always negative for neutralizing antibodies, antisera mixed with the various toxin preparations often induced precipitation. The only serum which failed to cause precipitation was that from chickens recovered from coccidiosis. Ring tests with serum from birds that had been injected twice with either ammonium sulfate-precipitated or oocyst extract showed that precipitating antibodies appeared after 4 days. These reached a maximum on the 6th day following the first injection.

Chickens injected with toxic extracts were not protected from a subsequent experimental coccidial infection.

A report of the physical properties of the toxic substance described herein must necessarily await purification of the toxin. It is possible that the toxic principle is attached to some protein molecule in the extract and properties attributed to treatments influencing the toxic activity may represent the properties of the protein moiety and not the toxin itself. The toxin in the crude cecal extract was precipitated in 40–55% saturated ammonium sulfate, whereas oocyst extract was precipitated below 40%. Mild heat (56° C for 15 minutes) destroyed the toxic activity of crude cecal and ammonium sulfate-precipitated extracts but the toxin in the oocyst extract was still present after 45 minutes at 56° C. Toxic activity was never diminished by dialysis. The extracts, kept at 4° C or frozen, retained their toxicity for over a year.

DISCUSSION

This report of a substance extractable from *Eimeria tenella* which is lethal to rabbits is apparently the first from among the coccidia. Bachman (1930a, b), studying immunity in rabbit coccidiosis, injected rabbits with dried oocysts (*E. steidae* and *E. perforans*) extracted with ether from infected rabbit liver and intestine. He mentioned no adverse reaction on the part of the rabbit to the coccidial preparations. Chapman (1929) extracted infected intestinal mucosa (*E. perforans*?) from rabbits in much the same way that crude cecal toxin was prepared in this study. She injected rabbits intravenously with 5 to 10 ml of the extract with no effect on that animal. She also attempted to induce antibody-formation to coccidial protein by injecting rabbits with dried oocysts, aqueous and alcoholic extracts from heavily infected intestine, and free sporozoites. None of these was reported to be particularly detrimental to the animal.

The toxin herein described closely resembles sarcocystin, a toxin long known to be extractable from sarcosporidian cysts. Sarcocystin was studied by many workers

following Pfeiffer's initial observations of its action (Pfeiffer, 1891). Much information regarding the nature of sarocystin can be found in reports by Laveran and Mesnil (1899), Rievel and Behrens (1904), Teichmann (1910), Teichmann and Braun (1911) and Sato (1930). The two extracts are similar in that rabbits are the only hosts which consistently respond to their toxic activity. The character of the symptoms and the time at which they appear in rabbits injected with either extract are similar, as are the post-injection times of death. However, toxin-neutralizing antibodies produced in rabbits injected with sublethal doses of toxin were much more consistently obtained by workers using sarcocystin than was the case with coccidial toxin. In the latter, neutralizing antibodies were obtained chiefly against the crude preparations (crude cecal extract, lyophilized cecal extract and, in one instance, oocyst extract). Further purification resulted in extracts still retaining their toxicity but lacking in ability to stimulate the formation of antibodies in the rabbit. Failure to obtain antibodies in chickens further complicates conclusions regarding the antigenicity of the toxin. It may be that the toxin in partially purified extracts simply was not antigenic in either the rabbit or the chicken. The non-toxic protein impurities present in the crude extracts may have acted as adjuvants for the toxic principle in cases where neutralizing antibodies were formed by rabbits. These same extracts in chickens, on the other hand, may possibly have possessed such a diversity of antigenic materials that antibodies directed specifically against the toxin failed to form in this animal. There is also the possibility that the toxic principle may be excluded by accompanying host protein from significant contact with antibody-producing cells of the chicken. Effective arguments regarding the antigenicity of this material must await its purification.

It is not yet known whether the toxin extracted from the infected cecum was excreted by intact coccidia or whether it was released upon lysis of the parasites. Certainly conditions within the cecum at the time when toxin is present in greatest concentration exclude neither possibility. Toxin from oocysts, however, was obtained only after the oocyst wall was ruptured, and very likely many spores contained within the oocysts were ruptured in the process. It was obtained from both sporulated and unsporulated oocysts, and therefore was apparently associated with most if not all stages in the development of the parasite. The storage stability of the toxin and its weak antigenicity are characteristic of endotoxins.

There is a remote possibility that the toxic reaction in rabbits injected with these extracts was due to an anaphylactic response to the introduction of *Eimeria* protein. Presumably, antibodies to coccidial protein may occur in rabbits that harbor *E. stiedae* or *E. perforans*. The experiments herein described do not exclude such a possibility, although indirect evidence suggests that anaphylactic shock was not involved. First of all, the injection of moderate amounts of coccidial extract was invariably fatal to the rabbit. The fatal syndrome in rabbit anaphylaxis is seldom this consistent, even in highly sensitive animals. Secondly, the symptoms which did appear as a result of the injection always developed slowly over a period of hours. Immediate reactions were never observed. Lastly, normal rabbit serum mixed with toxic extract never caused precipitation nor diminished the toxic activity of the extract.

There is as yet no evidence to show that this toxin plays any role in the syndrome of cecal coccidiosis. Bertke (1955) suggested that perhaps the diverse systemic effects of this disease reflected the presence of a toxic substance elaborated by or

through the action of the parasite. The coccidial toxin described in the present report was shown to have no gross effects on healthy chickens and could in no way consistently induce any of the symptoms characteristic of coccidiosis. During a coccidial infection, however, birds lose large amounts of blood, do not feed, are apparently partially unable to utilize their blood glucose, and are under severe stress in various ways. Conceivably the accumulation of toxin by the chicken before and during the crisis of the infection could influence the animal in its disease-altered condition.

It has been mentioned that a consistent post-mortem finding in rabbits given lethal doses of coccidial toxin was the altered condition of the intestinal tract. The small intestine was found to be filled with fluid and gas and seldom contained any solid food. The stomach was found always to be filled with food, and it seemed as if the pyloric sphincter did not allow any material to enter the small intestine. This may act somewhat like an intestinal obstruction which, in rabbits, causes death in about 24 hours (Dragstedt et al, 1919). Schildt and Herrick (1955) observed that the motility of the crop and cecum was markedly diminished in chickens infected with *E. tenella.* When the pharmacological properties of this toxin are better known, it may be determined whether or not the changes in intestinal functions in these two cases are in any way correlated.

SUMMARY

1. Extracts prepared from *Eimeria tenella* oocysts and from the ceca of chickens infected with this parasite were lethal to rabbits when injected intravenously or intraperitoneally.

2. The toxin was storage-stable and non-dialyzable.

3. Sublethal injections of toxic extracts into rabbits induced the formation of antibodies that neutralized the toxin.

4. Chickens injected with toxic extracts did not produce toxin-neutralizing antibodies nor were they protected from subsequent coccidial infection.

5. Serum from chickens recovered from coccidiosis did not neutralize the toxin.

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RESEARCH NOTE

THE EFFECT OF PIPERAZINE CITRATE ON HATCHING AND MIGRATING LARVAE OF *ASCARIS LUMBRICOIDES*

The effect of piperazine citrate (Antepar) was tested on 2 stages of *Ascaris lumbricoides*—the newly hatched larvae in the lumen of the intestine and the migrating stages in the tissues of the intestine, liver and lungs.

Forty male white mice 6 to 8 weeks old were each inoculated orally with 5000 infective *A. lumbricoides* eggs, the latter having been cultured for 6 weeks in 1% formalin after removal from worms obtained from human sources. For tests of the drug's effect on hatching larvae, 10 mice each were given 100 mg/kg of piperazine citrate orally 1 hour before inoculation and thereafter at hourly intervals for a total of 6 doses. For tests on the migrating larvae 10 mice each were given a single daily dose of 300 mg/kg intraperitoneally, beginning 24 hrs after inoculation and continuing for 7 days. The 2 groups of 10 mice that were used as controls were given an equivalent volume of distilled water in like manner to that of the drug. All mice were killed by drowning 10 days after inoculation.

The larvae observed in brain squashes of all mice were actively motile. Those counted in pepsin digests of the individual organs and carcass were nearly all dead, presumably due to the digestion process since squash preparations of the organs prior to digestion revealed active larvae. Three of the first-group control mice died with complete lung consolidation during the experiment. The means of the number and extent of the pulmonary hemorrhagic lesions using the Brown and Chan (1955, Am. J. Vet. Res. **16:** 613–615) classification for the hatching-larvae group were: test 2, controls 4. The means of the number of larvae recovered from each organ for test mice and controls respectively were: spleen 1 and 7, lungs 8 and 73, liver 18 and 63, brain 0 and 1, kidneys 0 and 5, muscle (carcass) 6 and 346, total 33 and 495. Equal numbers of hatched larvae, namely 4 to 12, were found in each direct saline smear examination of feces collected during the 4th and 8th hour after inoculation in both the test and control mice. For the migrating-larvae group (2 controls of which died during the experiment with complete lung consolidation) the range of the Brown and Chan lung classification was: test 2–4, controls 2–4. The means of the number of larvae recovered from each organ for test mice and controls respectively were: spleen 5 and 6, lung 37 and 32, liver 47 and 69, brain 0 and 0, kidneys 0.4 and 0, muscle 110 and 104, total 198 and 211.

In the summary of their studies on the effect of piperazine hydrochloride on migrating larvae of *Ascaris suum*, Brown and Chan (loc. cit.) stated that piperazine hydrochloride administered orally to mice before and after feeding them infective eggs entirely prevented the hatching larvae from invading the blood stream and the lungs. Piperazine citrate similarly tested on *A. lumbricoides* in the present study did not prevent migration of all larvae but decreased the number recoverable from the various tissues to only 7% of those recoverable from untreated controls. When given 24 hrs after inoculation, i.e. when the larvae had already reached the tissues, no appreciable effect was observed.—EILEEN H. PIKE, *Department of Tropical Medicine and Public Health, Tulane University Medical School, New Orleans, Louisiana.*

ATOXOPLASMA PADDAE (ARAGÃO) FROM SEVERAL SOUTH PACIFIC SILVEREYES (ZOSTEROPIDAE) AND A NEW ZEALAND RAIL*

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A malariological assignment in the tropical South Pacific in 1952–54, undertaken for the Royal New Zealand Air Force and supported by funds from the N. Z. Department of Scientific and Industrial Research, provided opportunities for a number of side projects. One of these concerned the blood parasites of birds, and in its course several new host and locality records for *Atoxoplasma* were obtained. These records are discussed herein, together with another resulting from collections made in Stewart Island, New Zealand, by the Animal Ecology Section of the N. Z. Department of Scientific and Industrial Research. My thanks are due to the authorities named above, to M. Louis Devambez of the South Pacific Commission, who obtained some of the New Caledonian material, and to my wife, who took part in all the field work.

SURVEY DATA

Blood films were taken from 137 birds of 43 species and subspecies in the British Solomon Islands Protectorate (Guadalcanal, Bellona, Rennell), the New Hebrides (Espiritu Santo, Aore, Futuna, Aneityum), the Belep Group (Art), New Caledonia and Fiji (Viti Levu). In all cases thin films obtained from the heart were fixed in methyl alcohol and stained with Giemsa. The smears were prepared immediately after the birds were shot. They were stained as soon as possible thereafter, usually on the evening of the same day.

Eight (6%) of the birds proved positive for *Atoxoplasma* on the basis of at least 1 hour's examination of each slide using 8× oculars and oil immersion. The hosts were from various parts of the study area, but belonged to a single family, the Zosteropidae, or silvereyes:—

Zosterops flavifrons flavifrons Gmelin. Mission Bay, Futuna, New Hebrides. February 20 and 21, 1953. (4/5).

Zosterops flavifrons majuscula Murphy and Mathews. Anelgauhat, Aneityum, New Hebrides. February 26, 1953. (1/1).

Zosterops lateralis griseonota Gray. Uitoé River, S. New Caledonia. December 6 and 7, 1952. (1/6). (Devambez).

Zosterops rennelliana Murphy. Te-Uhungango, Rennell, British Solomon Islands Protectorate. August 20, 1953. (1/1).

Woodfordia superciliosa North. Te-Uhungango. August 20, 1953. (1/3).

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Atoxoplasma was not detected in the films from 3 *Z. f. majuscula* shot at Aneityum on an earlier occasion (August 5, 1952), 2 *Z. f. brevicauda* Murphy and Mathews from Aore, New Hebrides (August 13, 1952), and 2 *Z. l. griseonota* from Tontouta, New Caledonia (November 18 and 20, 1952).

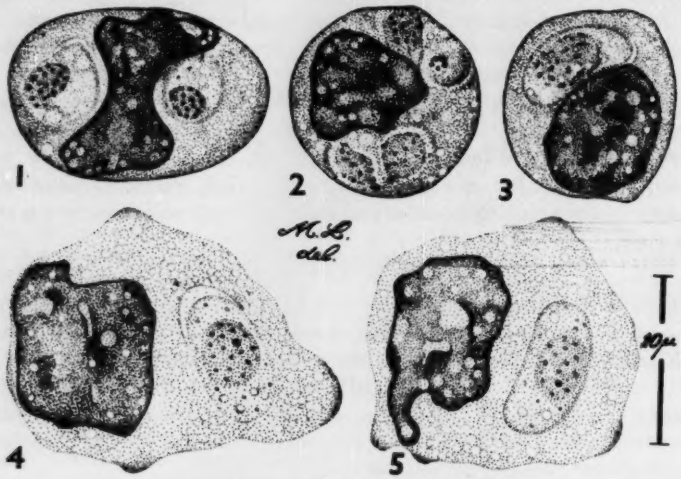
The remaining infection is recorded from a chick of the Stewart Island Weka, *Gallirallus australis scotti* (Ogilvie-Grant) (Rallidae), captured in December, 1950.

MORPHOLOGICAL ACCOUNT

(All dimensions in microns, and all averages derived from the measurements of 25 examples)

(Figs. 1–5)

Parasites from the various hosts closely resemble one another. The smallest forms are round (Fig. 2) to pyriform (Fig. 1), and measure from 2.5 to 4.3 (av., 3.2) in their greatest diameter. They occur in the cytoplasm of large lymphocytes, sometimes singly, but usually in twos (Fig. 1) and less often in fours (Fig. 2). Two such parasites commonly lie on opposite sides of the host cell nucleus, which becomes deeply indented (Fig. 1). Division is by binary fission, and no more than 4 daughter organisms have been seen within a single white cell.



Atoxoplasma paddae from various hosts.

FIGURES 1 AND 2. Division stages, lymphocytes of *Gallirallus australis scotti*, New Zealand.
FIGURE 3. Non-dividing form, lymphocyte of *Zosterops lateralis lateralis*, New Zealand.
FIGURES 4 AND 5. Non-dividing forms, monocytes of *Zosterops lateralis griseonota*, New Caledonia, and *Z. flavifrons flavifrons*, Futuna, respectively.

Larger stages occur singly in the cytoplasm of lymphocytes (Fig. 3) or monocytes (Figs. 4, 5), but do not indent the host cell nucleus. They are oval (Fig. 4) to sausage-shaped (Figs. 3, 5) bodies, usually with equally rounded ends but sometimes rather narrower at one end than the other. The nucleus, an oval structure the long diameter of which corresponds to that of the body, measures 3.0 to 5.9 (ave., 4.2) in length by 2.3 to 3.8 (ave., 2.9) in width. Most of the parasites on my slides are of this large type. Overall measurements from their several hosts are.—

6.1 to 8.4 (ave., 7.1) by 2.4 to 4.0 (ave., 3.1) (*Zosterops lateralis griseonota*)
4.9 to 9.1 (ave., 7.3) by 2.3 to 4.8 (ave., 3.3) (*Gallirallus australis scotti*)
5.7 to 9.5 (ave., 7.4) by 2.8 to 4.3 (ave., 3.6) (*Zosterops flavifrons flavifrons*)
5.1 to 10.7 (ave., 8.1) by 2.9 to 4.4 (ave., 3.8) (*Woodfordia superciliosa*)
6.4 to 11.1 (ave., 8.2) by 2.8 to 4.5 (ave., 3.7) (*Zosterops flavifrons majuscula*)
6.7 to 10.2 (ave., 8.4) by 2.6 to 4.8 (ave, 3.7) (*Zosterops rennelliana*)

Large and small stages react to Giemsa stain in similar fashion, the cytoplasm staining pale blue and the nucleus a faint pink with deeper pink granulation. The latter structure usually exhibits a single granule more intensely stained than the others (Figs. 4, 5). A number of tiny granules may be evident in the cytoplasm (Figs. 4, 5), which also contains a few small vacuoles. The absence of a distinct limiting membrane often renders it difficult to differentiate the parasite from the host cell cytoplasm in which it is embedded (Fig. 4), and in many lightly stained examples only the nucleus can be made out. In favorably stained examples the peripheral cytoplasm may assume a slightly deeper shade of blue than the remainder (Figs. 3, 5). This discloses the body outline, as does the common occurrence of a partial marginal halo (Figs. 1–4) presumably caused by slight shrinkage of the parasite during fixation.

Parasites from the different hosts and localities are of identical appearance, and slight disagreement in regard to average size is of small taxonomic value as is indicated by wide overlapping in size range. These facts, together with a complete lack of knowledge of life-history stages other than those occurring in heart blood, and the absence of host specificity data, are inadequate justification for the recognition of more than a single species at this juncture.

The organism is referred to the genus *Atoxoplasma,* which was established by Garnham (1950) for some avian parasites of uncertain standing previously referred to *Toxoplasma,* to which most modern authors (see Eyles and Frenkel, 1952) assign but one species, *T. gondii* (Nicolle and Manceaux, 1908). Birds, although certainly subject to infection with *T. gondii* (see Jacobs et al, 1952; Habegger, 1953), are more often parasitized by a superficially similar but basically very different organism which Garnham (1950) declared to be strictly host specific and non-pathogenic, and to possess a delicately granular cytoplasm not enclosed by a periplast, and a large diffuse nucleus with a tiny karyosome. Garnham's first 2 criteria are emended in the diagnosis presented hereunder. One change is made because Lainson (1958) has since demonstrated that *Atoxoplasma* may cause congestion and hemorrhage of the blood vessels in the liver and lungs and the development of inflammatory foci throughout these organs; the other because Garnham (1950) postulated strict host specificity from his failure to infect other animals by the inoculation of emulsions derived from heavily infected lungs. This hypothesis would carry weight were it established that *Atoxoplasma* is indeed closely related to *Toxoplasma,* to which no warm-blooded animal has thus far shown complete natural immunity (Lainson, 1958). However, toxoplasmas differ from the former genus in this and other respects, including their high degree of pathogenicity, their crescentic and heteropolar form and intense staining reaction with Giemsa, and the fact that repeated divisions by binary fission may lead to the production of as many as 150 to 200 organisms within a single cell. Present evidence suggests that the affinities of *Atoxoplasma* lie not with the toxoplasmas but with the haemogregarines (p. 51), and that failure to achieve transmission by subinoculation is due not to strict host

specificity but to the fact that the method employed has simply not resulted in the transfer of stages able to establish an infection in another vertebrate.

It is proposed to emend the diagnosis of *Atoxoplasma* as follows:

Benign to mildly pathogenic parasites, typically sausage shaped with more or less equally rounded ends; occurring in avian monocytes and lymphocytes, often causing pronounced indentation of the nucleus of the latter; their weak staining reaction and the absence of a well defined periplast rendering it difficult to differentiate their cytoplasm from that of the host cell; nucleus diffuse, granular and with a tiny karyosome; multiplication by binary fission, few (usually 2) division products per host cell; not transmissible by subinoculation.

SPECIFIC STATUS

It remains to determine the species to which this organism should be assigned. Marullaz (1913) proposed the name of *Toxoplasma avium* for a parasite of *Padda oryzivora* first discovered by Laveran (1900), and Garnham (1950) selected *Atoxoplasma avium* (Marullaz) as the type of his new genus. However, Aragão (1911) had preceded Marullaz in assigning a name, *Haemogregarina paddae*, to Laveran's parasite, and must be conceded priority as recognized by Wenyon (1926) and Herman (1937). Although Aragão merely added the formality of a name to the brief and incomplete account published by Laveran (1900) the latter author's data fit the dividing and early post-division stages of the organism which Marullaz (1913) described from the same host as *Toxoplasma avium*. Garnham (1950) thus selected a specific name inadmissible under Article 27 of the International Rules of Zoological Nomenclature, and *A. paddae* (Aragão, 1911) must therefore be regarded as the type of the genus *Atoxoplasma*.

All known members of this genus are indistinguishable from *A. paddae* in their morphology and effect upon the host cell. Rejecting the criterion of strict host specificity as not proven, we are left with little more than the factor of overall size as a guide in the recognition of species. In view of the range in length and breadth exhibited by the Pacific parasites, it is not considered that minor differences between average dimensions for strains from various birds can be regarded as more than a manifestation of the adjustment of a non-specific parasite to individual hosts. *A. paddae* commonly reaches a size of 5 to 6 by 2.5 to 3 (Marullaz, 1913), and these figures fall within the lower part of the range for Pacific material. In any case, the figures given by Marullaz were represented as a size frequently attained, not as an absolute range. This renders it obvious that too much significance should not be attached to their slight disagreement either with the Pacific averages or with the dimensions of 7.5 by 3 advanced, together with the claim of strict host specificity, as evidence of the distinctness of *A. argyae* Garnham, 1950.

It is submitted that only a single species of *Atoxoplasma* is at present recognizable, that the correct name for this species is *A. paddae* (Aragão, 1911), and that the organisms now recorded from New Zealand and the South Pacific belong here.

DISCUSSION

Toxoplasma-like parasites now referred to *Atoxoplasma paddae* have been reported from Australasia twice before, first by Lawrence (1946) from introduced sparrows at Sydney, New South Wales, and subsequently by Laird (1950) from *Zosterops l. lateralis* (Latham), an immigrant bird from Australia, at Masterton in the North Island of New Zealand. Although it was remarked that in examples derived from the latter host one end was often more pointed than the other (Laird,

1950), re-examination of the material indicates that the heteropolar condition is much less marked than in *Toxoplasma* and that there is general agreement with *A. paddae.*

A prior report of toxoplasmosis from a silvereye perhaps belongs here too, Rosenbusch (1932) having discovered a natural infection in *Zosterops palpebrosar peguensi* in Argentina.

It is evident from the literature that most records probably referable to *Atoxoplasma* concern passerine birds. There does not appear to be a previous instance of infection in a rail, and *Gallirallus australis scotti* is the first indigenous New Zealand bird to prove positive for *Atoxoplasma. Zosterops lateralis,* local races of which are found from New Caledonia to the Banks Islands and Fiji (Mayr, 1945), reached New Zealand from eastern Australia a little over 100 years ago. Since then it has succeeded in colonizing the Chatham Islands, Norfolk Island, the Kermadecs, and the Snares, Auckland, Campbell and Macquarie Islands (Fleming et al, 1953). Wide-ranging and migratory habits distinguishing some Northern Hemisphere Zosteropidae as well, it is interesting to speculate on the rōle that these birds may have played in the dissemination of a parasite to which they are so subject.

In conclusion, a suggestion is offered regarding the nature of *Atoxoplasma.* Members of this genus have often been identified as haemogregarines in the past. More specifically, Aragão (1919) contended that the parasite described by Marullaz (1913) was not a toxoplasma but rather a haemogregarine allied to *Hepatozoon;* and Reichenow (1953), pointing out that the morphology of *Atoxoplasma* combines with the failure of subinoculation experiments to prove that the organisms differ fundamentally from *Toxoplasma,* speculated that the former genus has affinities with *Schellackia* and *Lankesterella.* This hypothesis explains the inability to transmit *Atoxoplasma* by subinoculation on the plausible grounds that further development of the stages found in the bloodstream takes place in a bloodsucking invertebrate. In this connection, it should be noted that there is remarkable similarity between dividing forms of this genus and certain leucocytic stages of *Haemogregarina bigemina* Laveran and Mesnil. The latter invade lymphocytes and monocytes, develop into oval bodies having pale-staining cystoplasm and a diffuse nucleus, divide by binary fission, and often indent the nucleus of the host cell (Laird, 1953; Saunders, 1955). The division products are little vermicules which invade erythroblasts. Growth and a final division lead to the production of elongate gametocytes as the red cell matures. Two gametocytes are formed in each erythrocyte in the case of *H. bigemina,* larger numbers characterizing other members of the *bigemina* group. These haemogregarines parasitize marine fishes, and there is certainly no resemblance between their slender gametocytes, 2 or more of which occur in red cells, and the larger stages of *Atoxoplasma* found singly in white cells. Nevertheless, the close agreement between the lymphocytic division forms of these organisms deserves further study.

SUMMARY

It is pointed out that *A. paddae* is the correct name for the type species of *Atoxoplasma* Garnham, and that comparisons may be drawn between this genus of uncertain affinities and haemogregarines of the *bigemina* group. Reference to the literature indicates that *Toxoplasma*-like parasites already recorded from *Passer domesticus* in Australia and *Zosterops lateralis lateralis* in New Zealand are refer-

able to *Atoxoplasma,* which is now described from the following new hosts and localities: *Zosterops flavifrons flavifrons* (Futuna, New Hebrides); *Z. flavifrons majuscula* (Aneityum, New Hebrides); *Z. lateralis griseonota* (New Caledonia); *Z. rennelliana* and *Woodfordia superciliosa* (Rennell, British Solomon Islands Protectorate); and *Gallirallus australis scotti* (Stewart Island, New Zealand). It is held that our very incomplete knowledge of the genus does not justify the designation of new species at present. These organisms, being morphologically indistinguishable from *A. paddae,* are thus identified accordingly.

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A REVIEW OF THE REPTILE CHIGGERS, GENUS *FONSECIA* (ACARINA, TROMBICULIDAE), WITH DESCRIPTIONS OF TWO NEW AMERICAN SPECIES

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The genus *Fonsecia* was proposed by Radford (1942) to include 2 Brazilian chiggers, *Trombicula ewingi* Fonseca, 1932, the type species, and *T. travassosi* Fonseca, 1936. The principal generic character was the modified, stubby, peglike anterolateral scutal setae. A third species, *F. coluberina* Radford, 1946, extended the range of this group to India. The report of all 3 from only snakes suggested reptilian host specificity of *Fonsecia* larvae.

The distinctness of this group is well established, although authors have regarded it variously as a genus or as a subgenus of *Trombicula*. The larvae of the type species of *Trombicula, T. minor,* is unknown; however, Audy (1954) has presented convincing evidence that it is small, a parasite of bats, and closely related if not identical to one of several bat-infesting species in Malaysia. None of these appears to be related to *Fonsecia* species below the subfamilial level. Furthermore, 4 additional species, of which 2 new ones are described below, are now included in the group. All these are limited to reptilian hosts, and 2 are recorded from lizards as well as from snakes. For these reasons, therefore, we follow those authors (see under generic references below) who have accorded full generic status to the group.

The genus is redescribed as follows:

Fonsecia Radford, 1942
Type species: *Trombicula ewingi* Fonseca, 1932

Fonsecia Radford, 1942: 56; 1946: 247; 1954: 262; Womersley and Heaslip, 1943: 72; Hoffman, 1944: 48; Ewing, 1944: 347; 1949: 235; Finnegan, 1945: 58; Taylor and Murray, 1946: 147; Lawrence, T. J., 1947: 3; Lawrence, R. F., 1949: 469; Fuller, 1952: 15; Audy, 1954: 148; 1957: 56; 1957a: 244; Womersley and Audy, 1957: 266.

Trombicula (*Fonsecia*) Wharton et al, 1951: 28; Wharton and Fuller, 1952: 51; Womersley, 1952: 20.

Trombicula sens. lat. Gunther, 1952: 5.

Trombiculine larvae having a scutum with stubby, peglike anterolateral setae; branched, flagelliform sensillae; anterior setae set back from margin. Cheliceral blade with tricuspid cap, dorsal and ventral tooth; galeal seta long, nude. Palpal genual and laterotibial seta nude; palpal tarsus with 6 branched setae, a subterminala and a tarsala. Legs with 3 genualae I, tarsala I longer than tarsala II, no mastisetae III; only 1 branched seta on each coxa.

Members of the genus are reptilian parasites in humid subtropical to tropical climates of southeastern Asia and the Americas.

The genus can be subdivided into 2 groups, the "coluberina" group of 3 Asiatic species (*F. coluberina, F. ptyasi, F. celesteae*) and the "ewingi" group of 3 American species (*F. ewingi, F. anguina, F. palmella*). A unique New World species, *F. travassosi,* is poorly known and may be an aberrant fourth member of the "ewingi"

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group or may even represent a third group. The first group is characterized by a bifurcate palpal tibial claw and some body setae expanded basally, and the second group, by a trifurcate palpal tibial claw and no body setae expanded basally. *F. travassosi* differs from other American species by having a pentagonal scutum, and from all species by having the anteromedian scutal seta stubby and peglike.

Although we are hampered in determining true relationships among groups and species of chiggers due to insufficient knowledge of their biology and lack of associated larval with postlarval stages in the majority of forms, helpful clues are provided by larval morphology and host preference. Among American forms of *Trombicula* sens. lat., the "gurneyi" complex of 2 species and a subspecies (Loomis, 1955) as a group appears to be closest related to *Fonsecia* with which several morphologic and ecologic characters are shared.

Reptiles are the important hosts, although there are some additional records of the "gurneyi" group on a few birds and mammals. Habitats are similar, at least in the case of *T. gurneyi* Ewing and *F. palmella* n. sp. The short anterolaterals (not modified in "gurneyi" complex) and anteromedian are set back from the scutal margin; sensillae similarly branched; 3 genualae I, no mastisetae III; galeal seta nude, palpal genual, dorso- and laterotibial seta nude, palpal trsus with a subterminala; usual structures punctate; not many dorsal setae, about 20–34.

On the other hand, species of this complex are distributed largely in the temperate zone of United States and Mexico; the palpal tarsus has 5 branched setae, and tarsala I is equal to tarsala II.

Audy (1954) suggests the relationship of his "ilesi" group to *Fonsecia* without elaborating further, aside from the fact that *Trombicula ilesi* (Radford), itself from Africa, is parasitic on snakes.

Key to species of *Fonsecia*

1. AM seta modified, short and peglike (Brazil) ..... *travassosi*
   AM seta normal 2
2. PL and medial dorsal setae abruptly expanded basally; palpal claw bifurcate; sensillae set far ahead of level of PL's (Malaya) ..... *celesteae*
   PL and medial dorsal setae not abruptly expanded basally; palpal claw bifurcate or trifurcate; sensillae at level of or but slightly anterior to PL's 3
3. Dorsal palpal tibial seta branched (United States) ..... *palmella*
   Dorsal palpal tibial seta nude 4
4. Ventral palpal tibial seta nude; dorsal formula begins 2–6 (Brazil) ..... *ewingi*
   Ventral palpal tibial seta branched; dorsal formula begins 2–8 or 2–10 5
5. Palpal claw trifurcate; preanal setae not swollen basally (Guatemala) ..... *anguina*
   Palpal claw bifurcate; preanal setae swollen basally (India) 6
6. Eyes 2/2; AM seta slightly behind AL's ..... *coluberina*
   Eyes 1/1; AM seta slightly in front of AL's ..... *ptyasi*

ACCOUNTS OF SPECIES

The terminology used is largely that of Wharton et al (1951). We propose the new term "digituli" for the short terminal processes of the peglike scutal setae. These digituli form a crown or cluster which, in its most pronounced form, resembles a loosely-closed hand.

To avoid unnecessary repetition, a detailed redescription of the type species, *F. ewingi*, is given and abbreviated descriptions or redescriptions of the others are based on it.

Fonsecia ewingi (Fonseca), 1932
(Fig. 1)

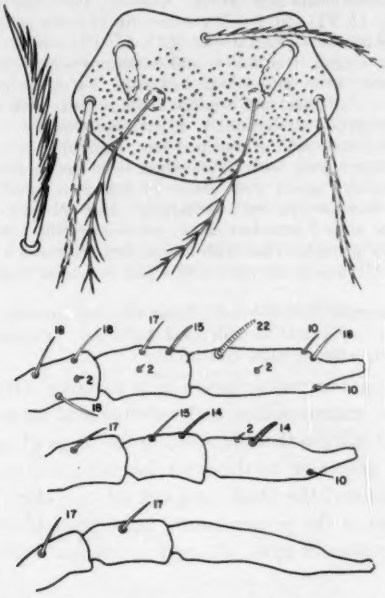

FIGURE 1. *F. ewingi.* Scutum, preanal seta, and specialized leg setae showing measurements in microns.

Trombicula ewingi Fonseca, 1932: 153; 1936: 30; Radford, 1942: 56; Rao and Hiregaudar, 1956: 210.
Eutrombicula ewingi, Ewing, 1938: 294.
Fonsecia ewingi, Torres and Braga, 1939: 38; Radford, 1942: 56, 76, 78; 1946: 247; 1954: 262; Fuller, 1948: 106; Audy, 1954: 148; 1957: 51; Rao and Hiregaudar, 1956: 212.
Trombicula (*Eutrombicula*) *ewingi,* Sig Thor and Willmann, 1947: 292; Fuller, 1948: 106.
Trombicula (*Fonsecia*) *ewingi,* Wharton and Fuller, 1952: 51.

Type data: Through the courtesy of Dr. Flavio da Fonseca, two cotypes were made available to us. Both specimens are in reasonably good condition. The one, here designated as the lectotype, bears the following data: "*Trombicula ewingi* Fonseca / capt. sobre *Ophis merremii* / de Correntes, M. Grosso / Flavio capt., mont. et det. / Phenol-Oleo de cedro / 23-IV-32 / Cotype." Virgules indicate end of each line on label. The other specimen bears similar data except that the date is "24-IV-32," and "oleo de cedro" is over-written with "Euparal."

We are redescribing the species as follows:

Diagnosis: F. ewingi may be separated from all other species of the genus by its nude palpal ventrotibial seta.

Body: Shape, ellipsoidal. Yellow in life (Fonseca, 1956). Length and width of lectotype, well engorged, 531 by 334 microns. Eyes 2/2, small, inconspicuous. Anus at level of 4th row of ventral setae.

Gnathosoma: Cheliceral bases and capitular sternum punctate. Cheliceral blade with small tricuspid cap and ventral projection. Palpal setae B/N/NNN/ (Audy, 1952; Traub and Audy, 1954). Palpal claw trifurcate. Palpal tarsus with 6 branched setae, a subterminala and tarsala. Galeal seta long, nude.

Scutum: Punctate, as figured. Less than 2 times wider than long. Corners not produced, posterior margin shallow convex. Sensillary bases at about level of posterolateral setae and separated by somewhat more than distance from either to its coresponding posterolateral seta. Sensillae flagelliform with about 12 long branches on apical two-thirds. Anterolateral setae short, stubby, with about 14 small digituli unevenly spaced on apical half. These modified

setae set well back from anterior margin of scutum. Remaining scutal setae of usual long, slender form with semi-appressed barbs. Anteromedian set back from margin but not as far as anterolaterals. Scutal measurements of lectotype: AW—50, PW—68, SB—29, ASB—26, PSB—16, AP—17, AM—46, AL—13, PL—50, S—57 (broken tip ?); of a paratype: AW—47, PW—66, SB—26, ASB—24, PSB—16, AP—15, AM—42, AL—14, PL—46, S—48 (broken tip).

Legs: Punctate. Leg I coxa, trochanter, and basifemur each with a branched seta; telofemur with 5 branched setae; genu with 4 branched setae, 3 long genulae, and a microgenuala; tibia with 2 branched setae, 2 tibialae, and a microtibiala; tarsus with about 16 branched setae; a long tarsala, a microtarsala, a subterminala, a parasubterminala (nude), and a pretarsala. Leg II coxa and trochanter each with a branched seta; basifemur with 2 branched setae; telofemur with 4 branched setae; genu with 3 branched setae and a long genuala; tibia with 6 branched setae and 2 tibialae; tarsus with about 14 branched setae, a tarsala (considerably shorter than tarsala I), a microtarsala and a pretarsala. Leg III coxa and trochanter each with a branched seta; basifemur with 2 branched setae; telofemur with 3 branched setae; genu with 3 branched setae and a long genuala; tibia with 6 branched setae and a long tibiala; tarsus with about 12 branched setae. All tarsi terminated with a pair of lateral claws, and a slender clawlike empodium between them.

Body setae: Dorsal formula: 2–6–6–4–2–2. Setae of approximately uniform length throughout the rows (± 46 microns). Ventral formula: 2–2–4–2–2–2–2. Postanal setae similar to dorsal setae. Preanal setae short, without basal constriction.

Discussion: In places, our redescription is at variance with the original description. We find that the anteromedian and anterolateral setae are farther removed from the anterior scutal margin than depicted in the original figure; that the anteromedian is anterior, not posterior, to the anterolaterals; that the branches of the sensillae are distributed around the shaft, and not on one side; that the sensillae are more nearly at the level of the posterolateral setae than anterior to this level.

Both anterior and posterior eyes, although small and indistinct, are discernible, not invisible.

In the setation of the legs, we differ from the original description as follows: Add the usual specialized microsetae and pretarsalae to legs I and II. Tibia I, add a tibiala; tarsus I, add a parasubterminala. Telofemur II, subtract a branched seta; genu II, add a genuala. Leg III, add tibia with 6 branched setae and a tibiala.

Apparent differences in dorsal and ventral formulae, but not in total numbers of setae, are due only to interpretation.

In passing, it is of interest to note that in detailing leg segmentation and setation, Dr. Fonseca had obviously recognized the value of the leg setae in the taxonomy of trombiculid larvae as early as 1932, 15 years before their revived diagnostic application by Wharton (1947) who later in 1948 proposed, for the specialized setae, the names which, for the most part, have now met general acceptance.

Fonsecia travassosi (Fonseca), 1936

Trombicula travassosi Fonseca, 1936: 47; Radford, 1942: 56; Rao and Hiregaudar, 1956: 210.
Trombicula travassoi (sic), Torres and Braga, 1939: 38.
Fonsecia travassosi, Radford, 1942: 76, 78; 1946: 247; 1954: 262; Fuller, 1948: 106; Audy, 1954: 148; 1957: 51; Rao and Hiregaudar, 1956: 212.
Eutrombicula travassosi, Ewing, 1938: 294.
Trombicula (*Eutrombicula*) *travassosi*, Sig Thor and Willmann, 1947: 292; Fuller, 1948: 106.
Trombicula (*Fonsecia*) *travassosi*, Wharton and Fuller, 1952: 51.

Type data: The holotype and only specimen of this species ever recorded has been misplaced or lost. However, we believe *F. travassosi* can be identified easily because of its distinctiveness. The unique specimen was collected by Lauro Travassos Filho from a snake, *Spilotes pullatus*, in Angra dos Reis, State of Rio de Janeiro, Brazil, presumably in December 1933.

The following account is based on the original description and on unpublished notes graciously supplied by Dr. Fonseca.

Diagnosis: F. travassosi is the only species of the genus having a modified peglike anteromedian scutal seta.

Body: Red in life (Fonseca, 1956). Length and width of holotype, engorged, 840 by 620 microns. Eyes 2/2. Anus between third and fourth rows of ventral setae.

Gnathosoma: Cheliceral blades broken. Palpal setae $B_7/N/NNB_4/$. Palpal claw bifurcate (?). Galeal setae broken.

Scutum: The original figure of the scutum was prepared with the aid of a camera lucida, thus its shape and placement of setae and sensillae should be fairly accurate. It is roughly pentagonal and approximately as wide as long. Puncta are numerous and generally distributed except for elongate clear areas behind the anterolateral angles. The anteromedian seta (13 microns), as well as the anterolateral setae (17 microns), is short and stubby and has several digituli at the tip. The posterolateral setae (57 microns) are of the usual form with short barbs. The sensillae, branched on their apical halves, are widely separated and at a level about midway between anterolateral and posterolateral setae.

Legs: From the information at hand, the setation of the legs is probably very similar to that of *F. ewingi.* There are 3 genualae I.

Body setae: Dorsal formula: 2–6–6–4–2. Ventral formula: 2–2–6–2–2–2. There appears to be only one pair of setae posterior to the anus.

Fonsecia coluberina Radford, 1946
(Fig. 2)

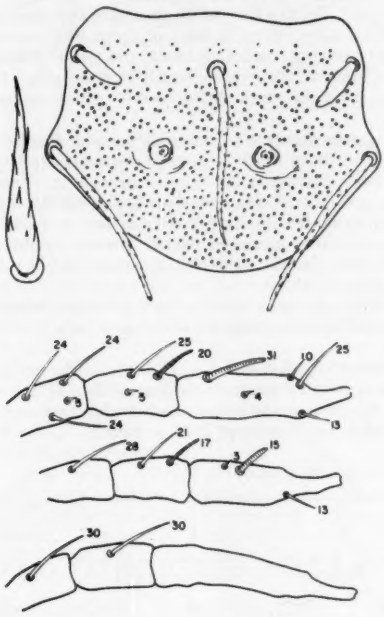

FIGURE 2. *F. coluberina.* Scutum, preanal seta, and specialized leg setae showing measurements in microns.

Fonsecia coluberina Radford, 1946: 249; 1954: 262; Lawrence, T. J., 1947: 3; Audy, 1954: 148; 1957: 51; 1957a: 244; Womersley and Audy, 1957: 266.
Trombicula coluberina, Womersley, 1952: 104; Gunther, 1952: 19.
Trombicula (*Fonsecia*) *coluberina,* Wharton and Fuller, 1952: 51.

Type data: Some years ago, one of us (J.M.B.) received 2 specimens of *F. coluberina* from Dr. Radford. These are from *Naia tripudians fasciatus* (=*Naja naja*), cobra, 19 May 1945, Imphal, Manipur, India. More recently, one of us (R.B.L.) also received from Dr. Radford a specimen off *Coluber radiatus* (=*Elaphe radiata*), copperhead racer, no date, same locality. Although Radford (1946) did not select a holotype, he referred to *Coluber radiatus* as the "type host." Presumably then, specimens recorded from it in the original description as "Imphal,

Manipur State. May 10th 1045" may be regarded as the type series. We have not seen specimens of this lot.

According to Radford, the unmodified scutal setae and ventral setae are simple, unfeathered. Womersley (1952), in a brief diagnosis of a "paratype" (syntype), states that all setae of the palpal tibia are nude, and that the dorsal setae are "to 30 microns long."

Our redescription, based on the specimens mentioned above, is not in complete accord with the original description or with the observations of Womersley. The ventral seta of the palpal tibia has 5 long conspicuous branches. The dorsal setae are 40 to 60 microns long. The unmodified scutal setae, ventral setae and dorsal setae are barbed, perhaps more definitely than noted by Womersley. In spite of these apparent or real differences, there seems to be little doubt as to the identity of our material. Within the limits of variation, the scutal measurements given by us agree with those of both Radford and Womersley; aside from interpretation, the dorsal formulae are similar, and the number of ventral setae correspond closely. Audy (1957) calls attention to the basal expansion of the preanal setae, a character very marked in our material.

It is regrettable that a holotype was not designated or that a lectotype has not been chosen, and more regrettable that we are not in a position to do so, since clearly a lectotype must be selected from the series off *Coluber radiatus*, 10 May 1945.

Diagnosis: This species is closely related to *F. ptyasi* Rao and Hiregaudar, from which it is separated by eyes 2/2 and AM slightly behind AL's.

Body: Shape, broad-oval. Length and width of a partially engorged specimen, 475 by 360 microns. Eyes 2/2, small, no plate. Anus at level of about fifth row of ventral setae.

Gnathosoma: Capitular sternum, cheliceral bases, and palpal femoral plate with large, profusely scattered puncta. Cheliceral blade with small tricuspid cap. Palpal setae B/N/NNB/. Palpal claw bifurcate, the short dorsoexternal accessory prong originates near the tip of the axial prong.

Scutum: With large, scattered puncta. Somewhat broader than long, posterior margin deep convex, corners rounded. Sensillary bases at level of posterolateral setae and separated by less than distance from either to its corresponding posterolateral seta. Sensillae with 6 branches on distal half. Anteromedian and anterolateral setae set back from anterior margin, former behind latter. Anterolateral setae short, broad, ~~digituli very~~ minute and not well-defined. Remaining scutal setae long, slender, with few appressed ~~barbs~~. Scutal measurements of two specimens: AW—67, 68; PW—86, 83; SB—26, 22; ASB—36, 35; PSB—30, 26; AP—26, 26; AM—48, 54; AL—14, 15; PL—44, 41; S——, 84.

Legs: Punctate. Number, structure, and distribution of specialized and nonspecialized setae as in *F. ewingi*. Setae generally much longer. Ratio of tarsala I to tarsala II is 2.0. Tarsala I very long (31 microns).

Body setae: Dorsal formula: ~~2-6-8-8-4-4-2~~. Length of dorsal setae varies from 40 to 60 microns, setae of posteriormost rows longest; anteromedials slightly swollen basally. Ventral setae 2-2 (sternals) plus 50, those posterior to anus similar to dorsal setae. Preanal ventrals (not including sternals) relatively short and swollen basally.

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Fonsecia ptyasi Rao and Hiregaudar, 1956
(Fig. 3)

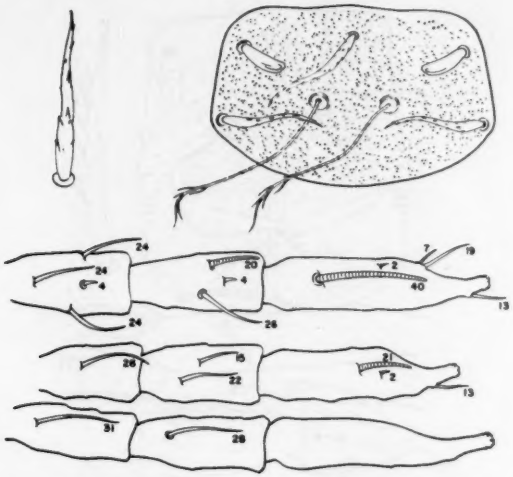

FIGURE 3. *F. ptyasi.* Scutum, preanal seta, and specialized leg setae showing measurements in microns.

Fonsecia ptyasi Rao and Hiregaudar, 1956: 210.

Type data: Through the kindness of Dr. Hiregaudar we have received 10 specimens of this species, presumably of the type series, off rat snake, *Ptyas mucosus,* Bombay Veterinary College Compound, India, June 1953. Although a holotype was originally selected and deposited in Bombay Veterinary College, the authors did not indicate the number of specimens in the series studied.

Diagnosis: F. ptyasi is closely related to *F. coluberina* from which it differs by having only a single eye on each side, more dorsal setae in the first row, a more shallow scutum, AM anterior to AL, and SB anterior to PL.

Body: Large, broad-ellipsoidal, a "paratype" well-engorged, 660 by 453 microns. Eyes minute, 1/1. Anus between sixth and seventh rows of ventral setae.

Gnathosoma: Cheliceral bases and capitular sternum conspicuously punctate, blades normal for the genus. Palpal setae B/N/NNB/; claw bifurcate, the minute accessory prong dorso-external and arising near tip of axial prong.

Scutum: Broader than long with rounded corners and shallow convex posterior margin, densely punctate. Sensillary bases anterior to level of posterolateral setae. Sensillae with several branches on apical half and minute barbs on basal half. Anteromedian and posterolateral setae slightly expanded basally. The stubby anterolaterals with few minute digituli. AM anterior to AL. Scutal measurements of a "paratype": AW—74, PW—92, SB—25, ASB—36, PSB—32, AP—28, AM—43, AL—15, PL—36, S—80.

Legs: Punctate. Number, form, and distribution of setae as in *F. ewingi.* Specialized setae much longer. Ratio of tarsala I to tarsala II is about 2.0. Tarsala I exceptionally long (40 microns).

Body setae: Dorsal formula: 2–(2–8)–10–6–4–4, 2–(2–8)–8–8–4–4, etc. First dorsal row with 10 setae, of which the medial pair are usually set forward, also they and other anterior dorsals are slightly expanded basally. Length 44 to 52 microns, the posterior ones longest. Ventral setae: 2–2 (sternals) plus 50. Preanals short and basally swollen.

Fonsecia celesteae Audy, 1957
(Fig. 4)

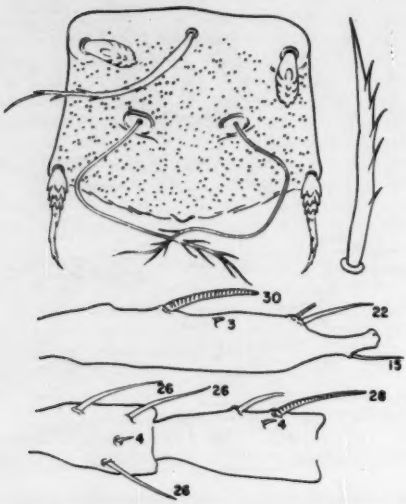

FIGURE 4. *F. celesteae.* Scutum, preanal seta, and specialized setae of leg I.

Fonsecia celesteae Audy, 1957: 49; 1957a: 244.

A detailed and adequate description of this species was given by Audy (1957). Our brief account, based on the original description and a paratype received through courtesy of Dr. Audy, is included merely for completeness.

Type data: "Holotype, CORU 3?961, and 2 Paratypes, ex snake R.31816 *Natrix trianguligera* (Boie) (Triangle Keelback), Bukit Lagong F. R., Kepong, Selangor, 18.iii.1953; and 1 Paratype ex skink, R.31608, *Mabuya multifasciata* (Kuhl) (Common Skink), Bk. Lagong F. R., Kepong, Selangor, 2.ii.1953. Holotype (BM. 1956.8.21.11) deposited with British Museum (Natural History), 1 paratype with U. S. National Museum, and 2 paratypes with the author's collection."

Diagnosis: "Differs from all known species of *Fonsecia* in having the posterolateral scutal setae and anterior dorsal setae conspicuously modified by peculiar basal swellings, the anteromedian scutal seta being normal."

Body: Ellipsoidal, yellow in life, holotype, partially engorged, 660 by 460 microns. Eyes 2/2, on a plate. Anus at level of 5th row of ventral setae.

Gnathosoma: Cheliceral bases and capitular sternum punctate. Palpal setae B/N/NNB/; claw bifurcate, the very short accessory prong dorsoexternal.

Scutum: Almost as long as wide, pentagonal, with obtuse posterior angle. SB anterior to PL. AM slightly anterior to AL. Sensillae with 10 or more branches on distal half. Large digituli of AL 12 or more, those arising from sides directed over inner hollow of seta. PL with abrupt triple-tiered basal expansion. Scutal measurements of a paratype (R.31816): AW—52, PW—70, SB—26, ASB—34, PSB—26, AP—32, AM—55, AL—14, PL—29, S—82.

Legs: Punctate. Setae approximately as in *F. ewingi* except for proportional lengths of specialized setae. Tarsala I (30 microns), tarsala II (20 microns), ratio: 1.5.

Body setae: Anterior and medial dorsals similar to posterolateral setae. Dorsal formula: 2-6-6-6-4-2. Ventral setae 2-2 (sternals) plus 16. Preanals not expanded basally.

Fonsecia anguina n. sp.
(Fig. 5)

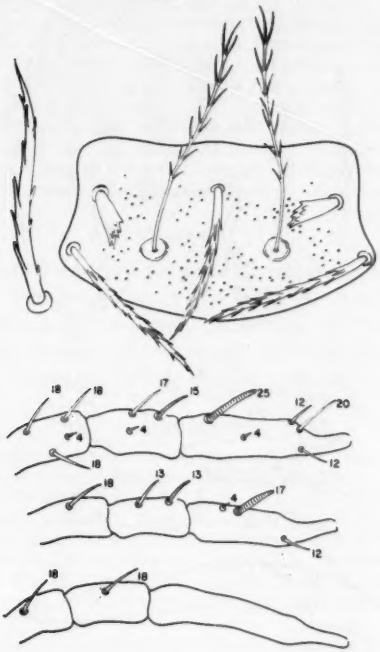

FIGURE 5. *F. anguina.* Scutum, preanal seta, and specialized leg setae showing measurements in microns.

Type data: Holotype and 2 paratypes, RML No. 28004, off "snake," Yepocapa, Chimaltenango, Guatemala, 12 November 1944, Herbert T. Dalmat, collector. Holotype and 1 paratype in the collection of the Rocky Mountain Laboratory, and 1 paratype in the United States National Museum.

Diagnosis: F. anguina is distinguished from its nearest relatives, *F. ewingi* and *F. palmella,* by 8 setae in the first dorsal row. From the former it is separated by a branched palpal ventrotibial seta and conspicuously larger terminal digituli of the anterolateral seta; from the latter by the larger ratio of the lengths of tarsala I and II, and by the larger scutum.

Body: Length and width of holotype, engorged, 558 by 475 microns. Eyes 2/2, small, indistinct, not on a plate. Anus at level of 4th row of ventral setae.

Gnathosoma: Cheliceral bases and capitular sternum punctate. Cheliceral blade with tricuspid cap. Palpal setae B/N/NNB/. Palpal claw trifurcate.

Scutum: Punctate, about 1½ times wider than long, corners not produced, posterior margin shallow convex. Sensillary bases slightly anterior to level of posterolateral setae and separated by more than distance from either to its corresponding posterolateral seta. Sensillae branched on apical two-thirds. Anterolateral setae short, stubby, palmate, set far back from anterior margin, 10 or more large and conspicuous digituli forming a terminal cluster. Anteromedian and posterolateral setae of usual form, with many appressed barbs, anteromedian set well back from anterior margin, but not as far as anterolaterals. Scutal measurements of holotype: AW—66, PW—81, SB—32, ASB—31, PSB—20, AP—19, AM—43, AL—14, PL—45, S—68 (?).

Legs: Punctate. Number, size, shape, structure, and distribution of specialized and nonspecialized setae essentially as in *F. ewingi.* Differences are negligible.

Body setae: Dorsal formula: 2-8-6-4-2-2. Setae of approximately uniform length throughout the rows (41 microns). Ventral formula: 2-2-4-2-2-4-2-2.

Fonsecia palmella n. sp.
(Fig. 6)

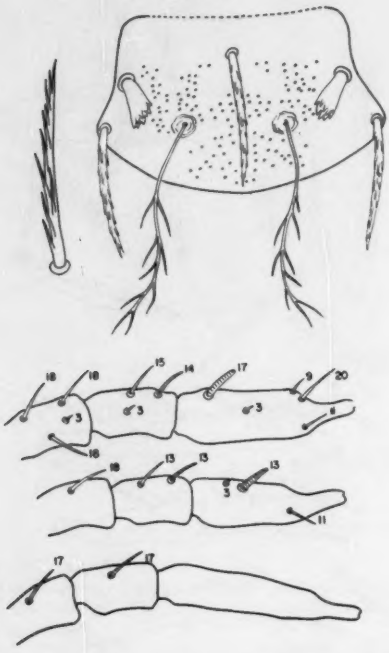

FIGURE 6. *F. palmella.* Scutum, preanal seta, and specialized leg setae showing measurements in microns.

Type data: Holotype and 7 paratypes (KU 3942–3949) from 2 southeastern five-lined skinks, *Eumeces inexpectatus* (field no. RL 540430–13), and 8 paratypes (KU 3950–3957) from 1 five-lined skink, *Eumeces fasciatus* (field no. RL 540430-13a), 8 miles northeast of Slidell, St. Tammany Parish, Louisiana, 30 April 1954, Richard B. and Margaret Loomis, collectors. Holotype and some paratypes in Snow Entomological Museum, University of Kansas. Other paratypes will be distributed to the Rocky Mountain Laboratory, U. S. National Museum, British Museum (Natural History), and to other selected institutions and individuals.

Diagnosis: F. palmella is distinguished from all species of the genus by a branched palpal dorsotibial seta, and in addition, from its nearest relatives, *F. ewingi* and *F. anguina,* by 6 setae in the 1st dorsal row combined with a branched palpal ventrotibial seta. Also in *F. palmella,* the ratio of the lengths of tarsala I and tarsala II is 1.3, in *F. ewingi* 1.6, and in *F. anguina* 1.5. These 3 form a compact group of species ranging from southern United States to Brazil. They are similar in shape of scutum, types of scutal setae, leg setation, body setae, and preanal setae without basal swelling.

Body: Length and width of holotype, engorged, 408 by 314 microns. Color in life, pale yellow. Eyes 2/2, small, red in life, ocular plate indistinct or absent, anterior larger than posterior.

Gnathosoma: Cheliceral bases and capitular sternum punctate. Cheliceral blade with dorsal tricuspid cap and a ventral tooth. Palpal setae B/N(B_2)/BNB/. Palpal claw trifurcate, with large axial and two short lateral prongs.

Scutum: Wider than long, angles not produced, posterior margin broadly curved, puncta small and scattered. Sensillary bases at level of posterolateral setae. Sensillae with 8–10 branches on distal half. Anterolateral setae short, stubby, palmate, set far back from anterior margin, with approximately 10 conspicuous digituli forming a terminal cluster. Anteromedian and posterolateral setae long, with the usual type of many appressed barbs, AM set back from anterior margin, but not as far as AL's. Scutal measurements of holotype: AW—57, PW—71,

SB—26, ASB—30, PSB—16, AP—14, AM—43, AL—12, PL—37, S—63; averages and extremes of 8 paratypes: AW—57 (55–60), PW—71 (68–73), SB—27 (25–28), ASB—30 (28–31), PSB—17 (16–19), AP—14 (13–15), AM—42 (39–44), AL—12 (11–14), PL—37 (36–40), S—63 (58–70).

Legs: Punctate. Number, size, shape, structure, and distribution of specialized and non-specialized setae essentially as in *F. ewingi.* Tarsala I somewhat longer than tarsala II.

Body setae: Dorsal formula, 2-6-6-4-2-2. Setae of approximately uniform length throughout (36 microns in anterior, 33 microns in posterior rows), humeral seta long (45 microns). Ventral formula, 2-2-4-2-2-4-4-2-2. Anterior and posterior sternal setae 35 microns, anterior and posterior ventral setae approximately 30 microns. Total number of body setae 46.

The specimens of *F. palmella* n. sp. were obtained by one of us (R.B.L.) while supported under a research grant E-547 from the National Institutes of Health to the University of Kansas Medical Center. This work was conducted under the direction of Dr. J. K. Frenkel.

SUMMARY

The 7 species of *Fonsecia* are all parasites of reptiles in tropical and subtropical climates; 3 occur in southeastern Asia, and 4 in the Americas. Of the latter, 2 are described as new. These are *F. anguina,* Guatemala, off "snake" and *F. palmella,* Louisana, off the skinks, *Eumeces fasciatus* and *E. inexpectatus.* The 5 others are redescribed. A key to species is included.

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DESCRIPTION OF *PARATRACTIS HYSTRIX* (DIESING, 1851) GEN. NOV. (NEMATODA: ATRACTIDAE) FROM *PODOCNEMIS DUMERILIANA*

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Nematodes of the sub-family Atractinae were obtained from the large intestine of the water turtle *Podocnemis dumeriliana*, collected at Pucallpa, Peru by Dr. L. Gonzales Mugaburu of the Zoology Department of the University of San Marcos, Lima, Peru. The specimens were fixed in 70% alcohol and cleared in lactophenol. The measurements of 200 specimens were made with the aid of a calibrated ocular micrometer and a microprojector.

Paratractis, gen. nov.

Diagnosis: Atractinae: Mouth surrounded by 3 well developed lips, 1 dorsal and 2 subventrals. Dorsal lip bears a basal pair and a submedian pair of papillae of the outer circle. Each of the subventral lips bears a basal papilla and an amphid and a submedian pair of papillae of the outer circle. Each lip near the oral opening bears a pair of papillae of the internal circle. Body cylindrical, attenuate at both ends; cuticle marked with fine longitudinal and transverse striations and covered with numerous scale-like cuticular projections, that alternate in diagonal rows. Esophagus divided into 2 portions, the anterior strongly muscular with the lumen chitinized and the posterior less muscular, ending in a large bulb. Cervical papillae present. Excretory pore posterior to the esophagus. Tail of males curved ventrally, bears 10 pairs of papillae; spicules unequal, and a short gubernaculum. Tail of females short, straight or slightly curved ventrally. Vulva near anus. Viviparous, 6–8 larvae. Adults parasites of turtles of genus *Podocnemis*.

Type species: Paratractis hystrix (Diesing, 1851).

Paratractis hystrix (Diesing, 1851)
(measurements in mm)

Description: Cuticle with transverse striations at regular distances of 0.001–0.003, specially noticeable at anterior extremity. Longitudinal striations form 2 dorsal and 2 ventral bands. With the exception of anterior end, cuticle bears numerous conic scale-like projections directed posteriorly; sparse and smaller at posterior end. Mouth surrounded by 3 lips, with 10 papillae and 2 amphids on external circle and 6 papillae on internal circle (Fig. 2). Esophagus: anterior portion muscular with rounded extremities and chitinized lumen; posterior portion narrow, less muscular and ends in a well developed bulb provided with 4 chitinous tooth-like plates (Fig. 3). Excretory pore opens posterior to esophagus and is surrounded by a chitinous ring connected to a muscular reservoir, where the 4 excretory canals empty (Fig. 1).

Male: 6.5–8 long by 0.37–0.50 wide. Esophagus: anterior portion 0.41–0.47 long by 0.10–0.11 wide: posterior portion 0.37–0.42 long by 0.14–0.17 wide. Cervical papillae and excretory pore at 0.27–0.34 and 1.18–1.37, respectively, from the anterior end. Cuticular projections begin at 0.13–0.16 from anterior end; there they are 0.036–0 053 long by 0.016–0.023 wide and increase gradually in number and size toward middle of body, maximum size being 0.09–0.10 long by 0.017–0.026 wide. They decrease in number on dorsal surface toward the tail where they are numerous in the adolescent, but sparse or missing in the mature specimen. On ventral surface these pro-

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The writer gave a former description of this parasite as *Paratractis spinifera*, in a thesis presented to University of San Marcos, Lima, Peru, in 1954.

jections form 2 unequal bands; a short right band composed of approximately 30 rows of projections and a long left band, composed of about 80 rows, which ends near anus (Fig. 6). Number of cuticular projects per row varies from 7–8 on proximal rows to 3–4 near anus; they are 0.02–0.03 long by 0.010–0.017 wide. Left spicule long and slender; length 0.37–0.42, width 0.023–0.030; transversally striated on its proximal half and ending in a sharp point. Right spicule short; length 0.17–0.18, width 0.017–0.023; narrows to distal extremity. Gubernaculum single: length 0.19–0.22, width 0.020–0.026; terminates in a barb (Fig. 7). Tail pointed, 0.21–0.29 long. Ten pairs of caudal papillae: 2 preanal, 3 adanal, and 5 postanal; 7 of these pairs ventral and 3 dorso-lateral (Fig. 8).

Female: 6.50–7.50 long by 0.38–0.51 wide. Esophagus: anterior portion 0.41–0.47 long by 0.10–0.11 wide; posterior portion 0.37–0.43 long by 0.14–0 18 wide. Cervical papillae and excretory pore at 0.27–0.36 and 1.19–1.39, respectively, from anterior end. Cuticular projections begin at 0.13–0.17 from anterior end; there they are 0.040–0.053 long by 0.017–0.023 wide, increasing in size toward the middle of the body, being 0.092–0.106 long by 0.020–0.026 wide, then decreasing in size toward tail, being 0.026–0.036 long by 0.010–0.013 wide near anus; not arranged in bands as are those of males. Single ovary connected to uterus which ends in a short muscular vagina. Vulva opens at 0.10–0.13 from anus. Tail 0.28–0.37 long with few cuticular projections on ventral surface (Fig. 4). Viviparous; mature female contains 6–8 larvae; no eggs have been observed.

Larvae: Body cylindrical, 2.25–2.70 long by 0.12–0.13 wide, covered with conic cuticular projections. Excretory pore at 0.54–0.60 from anterior end. Esophagus divided as in adults: anterior portion 0.25–0.26 long by 0.049–0.059 wide; posterior portion 0.18–0.20 long by 0.053–0.066 wide. Anus opens at 0.24–0.32 from posterior extremity (Fig. 5).

Host: Podocnemis dumeriliana.

Location: Intestine.

Geographic distribution: Pucallpa, Peru.

Type specimen: U. S. Nat. Mus. Helminth. Coll. No. 38005.

DISCUSSION

A review of the available literature concerning the subfamily Atractinae reveals that there are 2 previous descriptions of the nematode reported here. Diesing (1851), described briefly a new species of ascaroid, as *Ascaris hystrix*, based on the presence of 3 lips. Von Drasche (1882), studied the original specimens of Diesing and Molin and redescribed the species, under the name of *Atractis hystrix*, because of the divided esophagus, unequal spicules and the number of preanal papillae in the males. Although he recorded the presence of 3 lips he was unable to count the papillae of the external or internal circle. His redescription evidently is the one which has been consulted by subsequent authors.

According to Yorke and Maplestone (1926), and Gallego Berenguer (1947) 2 genera are to be considered in the sub-family Atractinae, Raillet, 1917: *Atractis* Duj., 1845, with 6 lips and *Leiperenia* Khalil, 1922, with more than 6 lips.

Of the 11 species described in the genus *Atractis*, 9 of them are described as having 6 lips. Travassos (1934) gave an incomplete description of *A. trematophila* and recorded the presence of 3 poorly developed lips each one bearing 2 papillae. Gallego Berenguer (1945) described *A. emilii* as having 3 bilobed lips, but his figure of the mouth shows 6 lips. If it is assumed that the bilobed lips of *A. emilii* are a misinterpretation of 6 lips, this brings up the question as to whether Travassos' description of *A. trematophila* is correct. Furthermore, he was not able to see the caudal papillae of the male. A redescription of this species may change its systematic position. In *Paratractis* there are 3 well developed lips with 10 papillae and 2 amphids on the external circle and 6 papillae on the internal circle.

The most striking features of the nematode here reported are the cuticular projections that cover the body in both sexes and in the larvae. Von Drasche mentions these projections but he does not describe nor show, in his figures, the special arrangement of these projections on the posterior end of the male. All the specimens

here observed possess 2 characteristic bands. These bands were not reported by Diesing nor von Drasche.

The number of caudal papillae reported for the male of *Atractis hystrix* is 22, while *Paratractis hystrix* has 20. This difference alone may not be sufficient to establish a new species.

The presence of 3 lips and the fact that the nematode is viviparous may place it in a more advanced position taxonomically than *Atractis*.

From the present description of *Paratractis hystrix*, it is evident while this nematode resembles the members of the genus *Atractis*, the presence of 3 lips, the arrangement of the cephalic papillae, and the cuticular projections that cover the body are sufficient grounds to separate it from the genus *Atractis*. Since it does not appear to fit into the genus *Leiperenia*, a new genus *Paratractis* has been proposed.

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Plate I

Explanation of Plate

All figures concern *Paratractis hystrix* (Diesing, 1851) gen. nov. Figure 2 was made with the aid of a camera lucida and other figures with a microprojector.

Figure 1. Anterior portion of female, showing the arrangement of cuticular projections and the excretory pore.

Figure 2. En face view of the head.

Figure 3. Anterior portion, showing the esophagus and papillae.

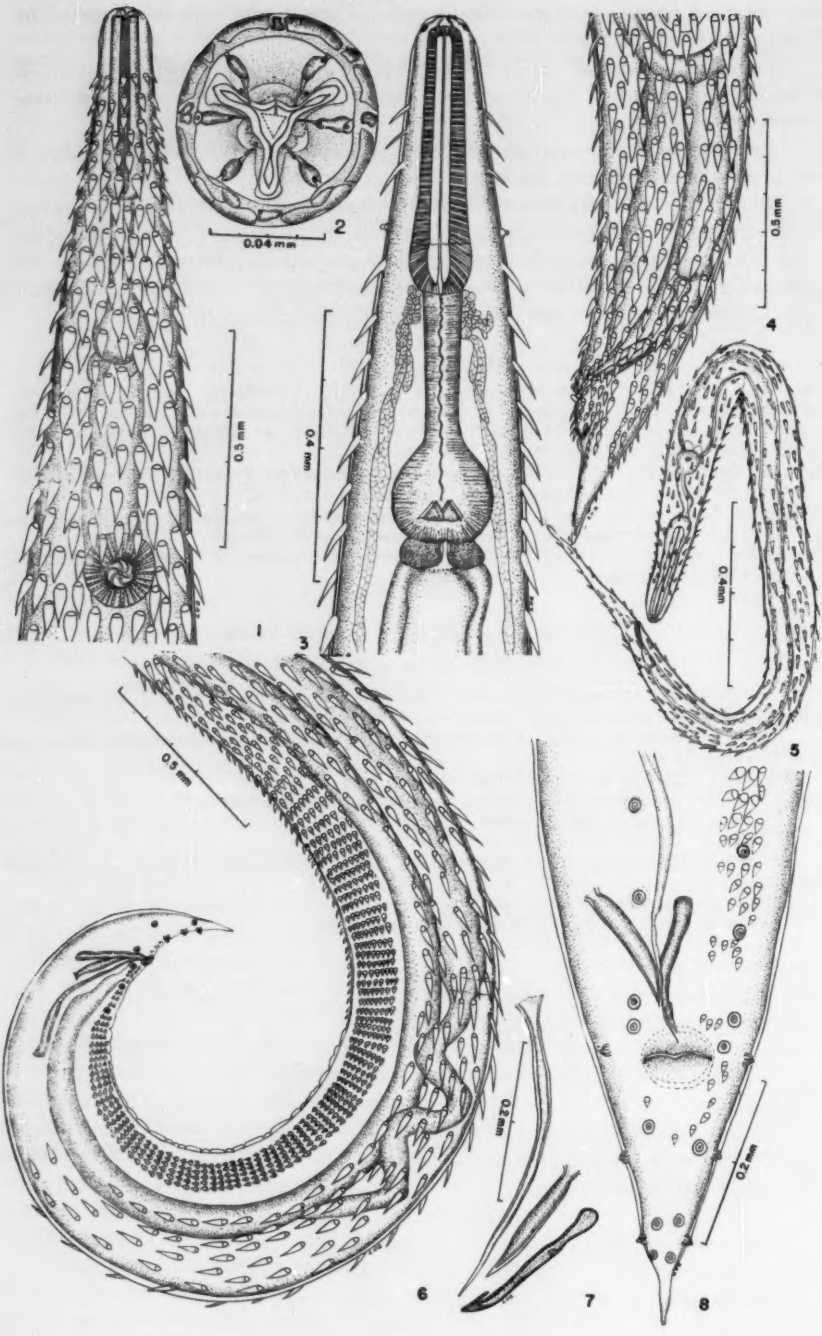
Figure 4. Posterior end of female. Lateral view.

Figure 5. Larvae, bearing cuticular projections.

Figure 6. Posterior portion of male, showing the left band of cuticular projections. Lateral view.

Figure 7. Spicules and gubernaculum.

Figure 8. Posterior portion of male, showing papillae.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MICROFILARIAE AND OTHER BLOOD PARASITES IN MEXICAN WILD DOVES AND PIGEONS

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During the winters of 1949 to 1952 the writer made smears of the heart blood of over 1600 wild birds from different parts of Mexico. Most of them were ducks and geese, but some were quail, songbirds, doves and pigeons. The microfilariae present in a number of these birds have already been described by Saunders (1955). The present paper deals with the blood parasites of a group of them which included 72 western white-winged doves (*Zenaida asiatica mearnsi*) and eastern white-winged doves (*Z. a. asiatica*); 38 western mourning doves (*Zenaidura macroura marginella*); 31 red-billed pigeons (*Columba f. flavirostris*); 1 Inca dove (*Scardafella inca*); and 3 white-fronted doves (*Leptotila verreauxi angelica*). The general purpose of the investigation was to determine which common blood parasites were present in these avian hosts.

MATERIALS AND METHODS

Blood was taken directly from the hearts of the freshly killed birds, and a thin film made of each. All slides were air dried, fixed in absolute methyl alcohol, and stained with Giemsa's stain. This stain was made by Hewitt's formula, and used according to his method (1940). Each slide was examined for 10 minutes under low power magnification for microfilariae, and again under an oil immersion objective for a similar period for the presence of other blood parasites.

Of the 72 white-winged doves collected, 14 were of the eastern subspecies and 58 were of the western form. The eastern birds were obtained near Taninul, San Luís Potosí, and Tuxtla-Gutiérrez, Chiapas. The western ones came from Ciudad Obregón, Sonora, and near Tepic, Nayarit. All of the red-billed pigeons, and the Inca dove, were obtained near C. Obregón, Son., as well as the mourning doves collected by the author. Two of the white-fronted doves were obtained at Iguala, Guerrero, and the third from Tuxtla-Gutiérrez, Chiapas. In addition to personally obtained specimens, Dr. Wilbur G. Downs, then of the Rockefeller Foundation in Mexico City, was kind enough to give the author the slides he had made and examined for blood parasites of a group of 8 western mourning doves collected by him at Acatlán (Chilapa), Gro.

RESULTS

The Inca dove and 3 white-fronted doves contained no microfilariae. Among the other doves and pigeons, microscopic examinations of the blood films showed 9 apparently different microfilarial forms which could consistently be differentiated by measurements and appearance. These were arbitrarily designated by the letters A through I, and comparative studies of them were made (Figs. A through I). Table I shows the measurements and characteristics of these microfilariae.

One eastern white-winged dove (7.1%), and 32 western white-winged doves (55.1%) were found to be infected with microfilariae. Only 1 microfilarial form (Fig. A) was found in the eastern whitewing. Its nuclei were tightly packed together, and there was a characteristic separation between the last several posterior nuclei, which were not continuous with those of the trunk.

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TABLE I.—*Characteristics of microfilariae from Mexican wild doves.*

Host	Length in Microns	Width in Microns	Rel. % Distance from Ant. End to Nerve Ring	Rel. % Distance from Ant. End to Excre. Pore	Rel. % Distance from Ant. End to 1st Genital Cell	Rel. % Distance from Ant. End to Anal Pore	Anterior End Shape	Posterior End Shape	Nuclei go to Anterior End	Nuclei go to Posterior End	Sheath	Position of Microfilariae	No. of Birds Examined	No. of Birds Infected
Eastern white-winged Dove Fig. A.	95.4–145.7; aver. 126.4	3.2	18.9	28.9	51.7	73.5	Round	Point	Yes or No	Yes	No	Highly twisted	14	1
Western white-winged Dove Fig. B.	58.3–110.2; aver. 90.4	2.6–4.7	16.5	35.9	49.9	60.1	Round	Round or Point	Yes	Yes or free space	No	Straight	58	11
Western white-winged Dove Fig. C.	175.0–233.2; aver. 206.8	5.3	18.1	28.0	46.4	64.4	Round	Point	Yes	No, 12% free	No	Gently curved or highly twisted		12
Western white-winged Dove Fig. D.	125.0–153.7; aver. 137.8	3.2–5.3	15.0	31.9	58.5	75.3	Round	Point	Yes	Yes	No	Straight		4
Western white-winged Dove Fig. E.	121.1–172.8; aver. 155.0	5.3	20.1	29.2	49.2	65.8	Round	Point	Yes	No, 11.5% free	No	Straight		5
Western mourning Dove Fig. F.	39.3–74.2; aver. 60.4	5.3	31.8	55.0	69.8	80.8	Round	Point	Yes	Yes	No	Lunate	46	3
Western mourning Dove Fig. G.	126.1–167.5; aver. 152.7	4.2	21.5	30.1	49.6	70.3	Round	Point	Yes or No	No, 5% free	No	Straight		2
Eastern white-winged Dove & Western mourning Dove Figs. A & G.	122.7–172.3; aver. 143.5	3.5–5.3	20.2	29.5	50.6	71.9	Round	Point	Yes or No	Yes or 5% free	No	Straight, curved, or highly twisted	60	3
Red-billed Pigeon Fig. H.	82.5–164.0; aver. 165.1	3.5–4.7	26.3	36.2	62.9	75.4	Round	Point	Yes	Yes	No	Straight or slightly curved	31	5
Red-billed Pigeon Fig. I.	159.0–208.8; aver. 182.4	5.3–6.3	11.9	29.3	38.2	55.1	Round	Round or Point	Yes	Yes	Yes	Highly twisted		2

Four microfilarial forms were found in western whitewings. The smallest one observed (Fig. B) was encountered in 11 birds. Its nuclei were very small and arranged loosely. They extended either to both ends of the body, or had a small free space at the posterior end. The caudal nuclei were continuous with those of the trunk. The largest form seen in western whitewings (Fig. C) was found in 12 birds. The caudal nuclei had considerable spaces between them and were not continuous with those of the trunk. Another form (Fig. D) was found in 4 western whitewings. Its nuclei were present to both ends of the body. Both the caudal and anterior nuclei had spaces between them and were not continuous with those of the trunk. The fourth microfilarial form (Fig. E) was found in 5 western whitewings. Its caudal nuclei had spaces between them, and were not continuous with those of the trunk.

Double microfilarial infections were found among 6 of the western white-winged doves. Two birds were infected with microfilariae as illustrated in Figs. D and E; 1 contained both C and E; 2 had both C and D; and 1 had both C and B.

Of the total of 46 western mourning doves from which blood smears were made by Dr. Downs and the author, 5 (10.8%) were found to be infected with microfilariae. Two forms differing greatly in appearance were found. One (Fig. F) was short and stout, and was present in 3 of the birds. Very large, densely packed nuclei were present to both ends of the body, and the caudal nuclei were continuous with those of the trunk. The other microfilaria of mourning doves was much longer and thinner (Fig. G) and was found in 2 birds. Its nuclei were much smaller than those of form F.

Of a total of 31 red-billed pigeons, 7 (22.6%) were found to be infected with microfilariae. Two very different forms were found in these birds. One form (Fig. H) was present in 5 of them. Its nuclei were small and present to both ends of its body, the caudal nuclei being continuous with those of the trunk. The second was present in 2 red-billed pigeons (Fig. I). It was large, stout, and sheathed. Its very large nuclei were present to both ends of the body, although the sheath was clearly visible in some larvae beyond the body ends. Its caudal nuclei were continuous with those of the trunk.

When the 9 microfilarial forms were first tentatively separated from each other on the basis of microscopic examination, it was suspected that several of them might prove to be the same species when the relative percentage distances to their major internal parts were studied (Table I). The percentage distances were so similar in some larvae that it was necessary to treat the data statistically to differentiate the populations accurately. F tests were run on every possible combination of the 9 tentative forms, using an analysis of variance according to Snedecor (1946).

Following Faust's (1949) statement of species differentiation whereby no 2 different species of microfilariae can have all 4 relative percentage distances alike, the statistical results indicated that Figs. B, C, D, E, F, H and I represented separate populations, but that Figs. A and G were identical forms and belonged to the same population. Forms B, C, D and E were all present in western white-winged doves; F was in western mourning doves, while H and I were in red-billed pigeons. Forms A and G were in both eastern white-winged doves and western mourning doves.

Haemoproteus sp. was found in 2 white-fronted doves, 38 western mourning doves, 13 eastern white-winged doves, 44 western white-winged doves, and 11 red-

billed pigeons. *H. columbae* was present in 2 western mourning doves, 3 western white-winged doves, and 1 red-billed pigeon. Following Manwell (1952) in his opinion as to the probable lack of validity of many named *Haemoproteus* species, all are considered as *Haemoproteus sp.*, except *H. columbae*, whose life history and vector are known. Dr. Carlton Herman kindly made those identifications involving *H. columbae*.

In *H. sp.*, the female gametocyte was very wide and usually rounded. It displaced the nucleus of, and at times enlarged, the red cell in which it occurred. The parasite measured 8.0 microns in length by 5.0 microns at its widest part. Its nucleus was usually found nearer the outer wall of the host cell, and was either centrally located or nearer one end of the gametocyte. The male gametocyte was longer and thinner than the female, and its rounded ends often curved around and displaced the host cell's nucleus. The nucleus of the male gametocyte was indefinite in outline, varied in shape, and usually centrally located. From 6 to more than 20 rod-shaped or round granules were present in both type of gametocytes.

Leucocytozoon infections were found in 2 western white-winged doves. They had rounded gametocytes and probably belong to *L. marchouxi* described by Mathis and Leger (1910), which has already been reported from the eastern mourning dove, *Zenaidura macroura carolinensis*, by Levine (1954). *L. sp.* has been recorded by Wood and Herman (1943) from western mourning doves from the southwestern United States. No *Plasmodium* or trypanosomes were found.

DISCUSSION

Although microfilariae are often found in birds, very little is as yet known about their pathogenicity or taxonomic relationships in these hosts. Most writers have merely indicated their presence or absence. Only minor attempts have been made to differentiate one from another on the basis of such characteristics as pointed or blunt ends, straight or curved positions, nuclei extending to or absent from the body ends, and measurements of overall length and width.

As has been shown by Saunders (1955) these characteristics do not accurately differentiate microfilarial forms. A statistical analysis of variation made by her on a group of microfilariae found in 2 species of songbirds from Mexico, showed one microfilarial population to contain all of these varying conditions. The same form may vary in length and width at different stages of its development; so measurements of these 2 dimensions alone, do not accurately demonstrate 2 populations.

At present, the differentiation of microfilarial forms into separate species rests mainly upon data presented by Faust (1949) in connection with his work on human microfilariae. He made a series of measurements from the anterior end of a microfilaria to the beginning of each of its 4 major internal structures, and expressed these distances in terms of their relative percentage of the total length of the larva, from its anterior end, saying, "The relative distances of these landmarks from one another along the longitudinal axis, together with the size and relation of length to breadth, are utilized in specific diagnosis . . . since they are constant in the same species."

Chandler (1952) followed Wehr's (1935) classification of the nematode superfamily, Filarioidea. Chandler said that with very few exceptions, all of the filarial species parasitic in man or domestic animals belong to the family Dipetalonematidae. He pointed out that many human filarial infections are practically impossible to

diagnose except by their microfilariae, and mentioned 7 different species, described as belonging to the genus *Microfilaria*, which are found in 1 species of host animal, namely man. The statistical analysis of the microfilariae found in Mexican western white-winged doves, based on measurements made by Faust's method, showed that there are 4 different microfilarial populations in this 1 avian host species. This fact indicates the need for caution in assigning any microfilaria as the larval form of any adult nematode, or vice versa, when only one or the other is encountered at a time in a host.

SUMMARY

Heart blood smears were made and examined for blood parasites from the following Mexican birds: 58 western white-winged doves, 14 eastern white-winged doves; 46 western mourning doves; 31 red-billed pigeons; 3 white-fronted doves, and 1 Inca dove. The Inca dove contained no blood parasites.

Microfilariae were found in 32 western white-winged doves, 1 eastern white-winged dove, 5 western mourning doves, and 7 red-billed pigeons. Measurements of what appeared to be 9 different microfilarial forms were made, from their anterior ends to the beginning of each of their 4 major internal landmarks. A statistical analysis of variation of these measurements resulted in the probability that there were 8 separate populations present. Of these, 4 populations were found in western white-winged doves, 1 in western mourning doves, 2 in red-billed pigeons, and 1 in both eastern white-winged doves and western mourning doves. Double infections with 2 microfilarial forms were found in 6 western white-winged doves.

Haemoproteus sp. was found in 2 white-fronted doves, 38 western mourning doves, 13 eastern white-winged doves, 44 western white-winged doves, and 11 red-billed pigeons. *H. columbae* was present in 2 western mourning doves, 3 western white-winged doves, and 1 red-billed pigeon. Two infections with what was probably *Leucocytozoon marchouxi* were encountered in western white-winged doves. No *Plasmodium* or trypanosomes were found.

ACKNOWLEDGMENTS

Thanks are due Dr. Carlton Herman, Wildlife Pathologist, U. S. Fish & Wildlife Service, for kindly providing the first quantity of Giemsa stain used, and for checking a number of the author's slides to corroborate identification of *Haemoproteus* and *Leucocytozoon*. Appreciation is also expressed to Dr. Wilbur G. Downs for his aid one winter in the use of his laboratory facilities in Mexico City, and assistance in parasite identification. Thanks also go to Dr. George B. Saunders, Research Biologist, U. S. Fish & Wildlife Service, the author's husband, and to many hunters for aid in collecting the birds obtained; and to Dr. Myron J. Willis, Chief, Field and Laboratory Statistics, Communicable Disease Center, Atlanta, Ga., of the Dept of Education, Welfare and Public Health, for assistance in the interpretation of some of the F test values obtained.

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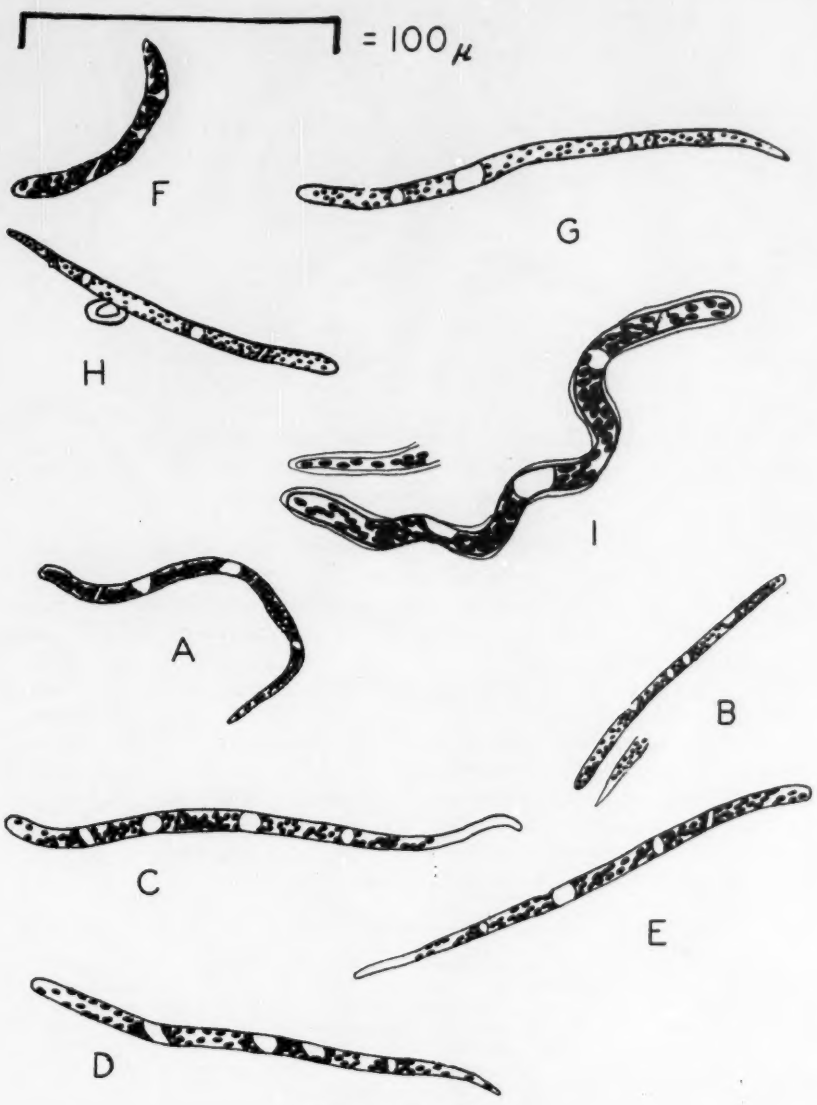

EXPLANATION OF PLATE

(All drawings were made with the aid of a camera lucida.)

FIGURE A. Microfilaria from eastern white-winged dove.
FIGURES B through E. Microfilariae from western white-winged dove.
FIGURES F and G. Microfilariae from western mourning dove.
FIGURES H and I. Microfilariae from red-billed pigeon.

EXPERIMENTAL STRONGYLOIDIASIS IN SHEEP AND GOATS. II. MULTIPLE INFECTIONS: DEVELOPMENT OF ACQUIRED RESISTANCE

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The writer (1955, 1959) previously reported that lambs and kids were clinically affected when experimentally exposed to single, cutaneous applications of large numbers of infective larvae of *Strongyloides papillosus*. Animals exposed to 100,000 or more larvae usually died, whereas others exposed to fewer than 100,000 larvae developed nonfatal clinical infections. The purpose of this paper is to report the results of an experiment on the resistance to challenge of lambs and kids previously exposed to immunizing infections, which the writer (1956) previously reported only in abstract.

Some evidence of acquired resistance to infection with *Strongyloides* spp. in various animals has been reported by a few workers. Sandground (1928) stated that once an infection of *S. stercoralis* was established in dogs it conferred a very effective and lasting immunity. Sheldon (1937a, b) also was successful in inducing an acquired immunity in rats to *S. ratti* after serial injections of small doses of infective larvae, and also reported the acquisition and retention of an almost absolute resistance after one previous infection was allowed to run its course and disappear. Later (1937c) he reported a sudden loss of *S. ratti* from rats 30 days after infection, which he believed indicated the development of acquired resistance. Enigk (1952a) concluded that acquired immunity prevented reinfection of adult sows with *S. ransomi*. Turner and Wilson (1958) reported the spontaneous cessation of an epizootic of strongyloidiasis in a lamb flock, which may have been due, in part, to the development of acquired resistance.

EXPERIMENTAL PROCEDURES

The 11 Shropshire lambs and 3 Toggenburg goats used in this investigation were raised parasite-free and were maintained in an isolation building in the same manner as has been previously described by Turner (1959). The techniques used in culturing infective larvae, inducing experimental infections, making the clinical observations, and recovering the parasites were also the same as those described in that paper. The feces were examined for eggs of *S. papillosus* daily for the first 3 weeks of the experiment and twice a week thereafter. All experimental animals were weighed at weekly intervals.

Eight lambs, 9 weeks old, were selected for the experiment to determine the degree of resistance elicited by repeated artificial percutaneous exposures to larvae of *S. papillosus*. These animals were kept in pairs in clean pens in the isolation building mentioned above. Six of these lambs were exposed to infective larvae of *S. papillosus* as shown in Table I and 2 served as un-

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TABLE I.—*Data on challenged and unchallenged lambs kept in pens and exposed to 10 immunizing percutaneous applications of infective larvae of* S. papillosus *and their respective controls.*

No. Lamb	Age and Weight at Time of Initial Infection		Immunizing Exposures* No. of Larvae		Age and Weight at Time of Challenge		Challenge Exposure No. of Larvae	Interval Between First Exposure or Challenge and Autopsy (days)	*S. papillosus* Recovered		
	(days)	(lb.)	(per exposure)	(total)	(days)	(lb.)			No.	% of Larvae Administered	Average %
					Challenged Lambs						
642	61	20	10,000	100,000	133	42	300,000	33	4,188	1.05	
617	60	24½	20,000	200,000	132	43½	300,000	33	8,028	1.61	1.03
623	60	22½	30,000	300,000	132	42½	300,000	33	2,543	0.42	
					Unchallenged Lambs						
612	61	20	10,000	100,000	133	33½	—	105	980	0.98	
616	61	23	20,000	200,000	133	33½	—	105	1,896	0.95	0.96
602	62	27	30,000	300,000	134	44½	—	105	2,827	0.94	
					Controls						
649	56	23½	—	—	128	43½	300,000	33	32,940	10.98	10.98
648	56	22½	—	—	128	50	—	—	0	0.0	

* Lambs exposed every other day.

infected controls. About 10 weeks after initial infection and 7½ weeks after the last exposure to larvae, single exposures of 300,000 larvae were administered to one member of each pair of previously infected lambs, and to one of the controls. This dose was selected because it normally produced severe, often fatal, infections in parasite-free lambs and kids. After the final exposure to larvae, the challenge-control lamb was separated from the uninfected lamb.

For the study of the development of resistance to this parasite on pasture, 3 parasite-free lambs similar in age and weight to those used in the experiment just described and 3 parasite-free kids approximately 6 weeks old were used. Two lambs and 2 kids were exposed to 30,000 larvae of *S. papillosus* (Table II). The remaining lamb and kid were not infected. The infected animals were transferred to a ¼-acre clean pasture of bluegrass, redtop, and mixed wild grasses. The uninfected control animals were put on a ⅛-acre clean pasture of the same type and quality as the pasture occupied by the principals.

Both pastures had been rested for a year, and the long drought of the previous year had aided in their parasitological decontamination. In addition all possible precautions were taken to prevent extraneous contamination of these areas before and during the course of the experiments.

In order to assure heavy contamination of the pasture occupied by the principals, approximately 10 pounds of fecal material containing large numbers of the eggs of *S. papillosus,* which were obtained from experimentally infected donor lambs, were distributed over the lot during the first week of the experiment. The infected animals were thus naturally exposed to larvae of *S. papillosus* which developed from the eggs distributed on the lot as well as from those passed in their own droppings. The latter were particularly numerous during the last 7½ weeks of the 9-week period on the pasture. The 2 controls were not exposed to infection.

TABLE II.—*Data on lambs and kids exposed to infection with* S. papillosus *on pasture during a 65-day period and subsequently challenged and their respective controls.*

Animal No. L-Lambs K-Kids	Age and Weight at Time of Initial Infection		Initial Infection (No. of Larvae)	Age and Weight at Time of Challenge		Challenge Exposure (No. of Larvae)	Interval Between Challenge and Autopsy (days)	*S. papillosus* Recovered		
	(days)	(lb.)		(days)	(lb.)			No.	% of Larvae Administered	Average %
L613	61	20½	30,000[1]	126	30	300,000	26	2,900	0.88	2.9
L621	60	25	30,000[1]	125	29	300,000	26	13,608	4.10	
K282	41	15	30,000[1]	106	24	300,000	20	4,400	1.30	
K283	41	17	30,000[1]	106	27½	300,000	20	17,700	5.30	
L603[2]	62	23	None	127	31½	300,000	26	82,338	27.50	48.05
K280[2]	41	15	None	106	28½	300,000	14	205,850	68.60	

[1] Plus continuous exposure to infection on pasture after first 2 weeks.
[2] Controls: Both died of strongyloidiasis; others killed.

At the end of the ninth week each animal was tested for susceptibility or resistance to infection with *S. papillosus* as was done in the preceding experiment by a single exposure to 300,000 larvae (Table II).

RESULTS

Multiple Infections of Lambs in Pens.

The average weight gained by the pairs of infected lambs and their controls is shown in Figure 1A. There was a slight retardation in the growth of the infected

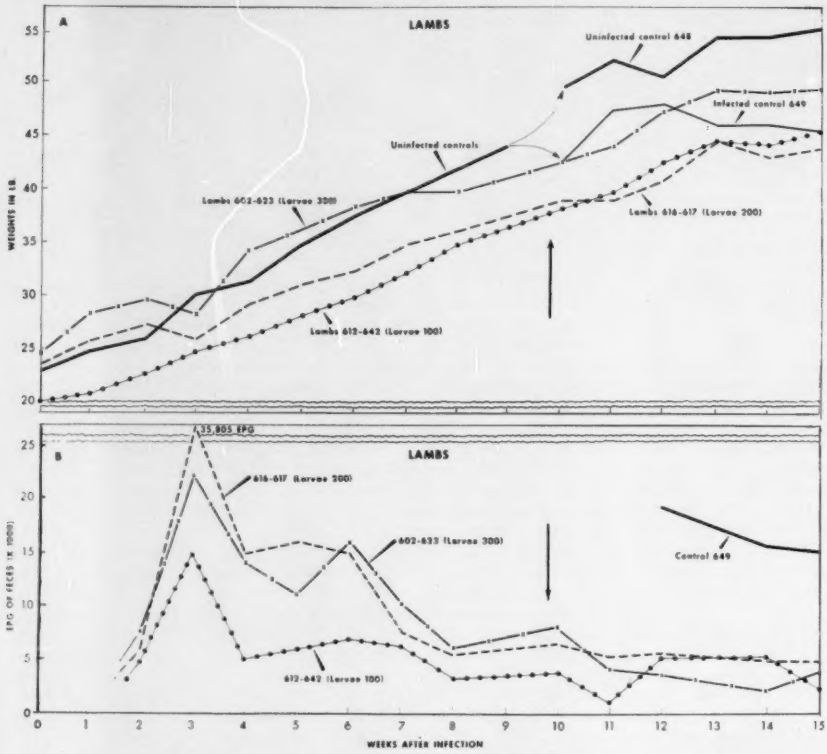

FIGURE 1. Multiple-infected lambs in pens. Arrows indicate when animals receiving initial infection were challenged and control was infected with 300,000 larvae. Larvae in thousands. **A.** Average weights of pairs of lambs similarly infected and controls. Control lambs after challenge shown separately; other pairs averaged after challenge because differences were minor. **B.** Average weekly egg counts for pairs of infected lambs. Egg counts first recorded for challenge-control lamb 2 weeks after challenge. As control 648 remained uninfected, there was no egg count from this animal.

animals before they were challenged. The pair receiving 100,000 larvae gained 17½ pounds, the pair receiving 200,000 gained 14¾ pounds, and the pair receiving 300,000 larvae gained 18¾ pounds, whereas the uninfected controls gained 23¾ pounds, or 5 to 9 pounds more than the principals.

The infected lambs after challenge either equaled or surpassed their unchallenged or uninfected controls in weight gains, with the exception of one pair. Challenged lamb 617 gained only 3 pounds during the postchallenge period, whereas its unchal-

lenged control, lamb 616, gained 9½ pounds. The average weight gained by the challenged lambs was 6¾ pounds, and that by the unchallenged lambs was 7 pounds. The uninfected control, lamb 649, which was initially infected at the time the principals were challenged, gained only 2½ pounds during the postchallenge period, compared with the 7½-pound gain of the remaining uninfected control lamb.

A slight-to-marked skin reaction at the inguinal site of larval application first appeared in all lambs on the fourth exposure to larvae 6 days after initial exposure. At this time pink areas were present in the inguinal region, and upon application of additional larvae a marked erythema developed. The erythema was particularly noticeable when the larvae were repeatedly applied to one area of inguinal skin, the rest remaining unexposed. After the sixth application of larvae, lamb 612 developed a slight erythema, which became more apparent 3 hours later. In this lamb the area of skin penetrated by the larvae was sensitive to touch and remained so for several days after the completion of the series of larval applications. In the other animals, however, only a slight reddening appeared, with no increase in intensity at the end of 3 hours. Urticaria was noticed after the seventh larval application in the 4 lambs receiving the smaller numbers of larvae. The wheals measured up to 3 cm in diameter and were accompanied by many pustules. Of the lambs exposed to a total of 300,000 larvae, no urticaria appeared, except in lamb 602 after the ninth larval application, and only varying degrees of erythema was seen in lamb 623 at any time. The sensitivity apparently was generalized, as erythema and/or urticaria appeared in both new and old exposure sites of the infected animals.

A marked erythematous reaction accompanied by urticaria was elicited by the challenge infections, the urticaria occurring within 2 hours after exposure. A day later the skin reaction was only slightly evident in lamb 642, and moderately persistent in lambs 617 and 623. No such reaction was observed in the parasite-free control lamb exposed for the first time to larvae of *S. papillosus*, or in any other lambs previously exposed to infection for the first time in the course of similar investigations.

The average numbers of eggs of *S. papillosus* per gram of feces passed by each pair of experimentally infected lambs and by the infected control lamb during each week of the experiment are recorded in Figure 1B. These data show that the eggs of this parasite appeared in the feces 9 to 10 days after the initial exposure to infection. The maximum number of eggs was passed approximately 3 weeks after the initial infection. The number of eggs then gradually decreased and was relatively small at the time the lambs were challenged.

The maximum number of eggs per gram of feces (EPG) passed by either of the lambs exposed to 100,000 larvae was 17,490; to 200,000 larvae, 62,520; and to 300,000 larvae, 29,490. These maxima occurred 22, 20, and 26 days after the initial infection, respectively, and were lower than those observed by the writer (1959) in a previously reported experiment in which the lambs received comparable numbers of larvae in single exposures. Maximum egg production occurred later in the lambs exposed to multiple infections than in lambs exposed to comparable single infections.

The numbers of worm eggs per gram of feces of the 3 previously infected lambs (642, 617, and 623) at the time they were challenged were 5,790, 9,060, and 6,300, respectively. The numbers passed at this time by the 3 lambs which were not challenged (612, 616, and 602) were 2,010, 4,200, and 9,720 EPG respectively. No

marked changes occurred in the number of eggs passed by the lambs after they were challenged with 300,000 larvae. The number of worm eggs passed by lamb 642 increased to only 10,800 EPG 3 weeks after challenge and then decreased to 4,800 EPG one week later. The number of worm eggs passed by lamb 617 did not increase until 5 weeks after challenge when only 10,000 EPG were recorded. The number of worm eggs passed by lamb 623 did not increase, but continued to decline until it was 750 EPG 4 weeks after challenge. The number of worm eggs passed by the unchallenged members of each infected pair of lambs continued to decrease to low levels, except that of lamb 602, which decreased only slightly to 7,440 EPG.

On the other hand, the number of eggs passed by challenge-control lamb 649, which received an initial dose of 300,000 larvae simultaneously with the challenged principals, increased to a maximum of 20,000 EPG 12 days after infection. The feces of uninfected control lamb 648 remained free from worm eggs throughout the experimental period. No abnormal stools were noticed in any of the animals during this time.

A slight decrease in hematocrit readings and hemoglobin values in the infected animals was noted during the first 9 weeks after initial larval exposure. The greatest hematocrit decrease, 36 to 25%, occurred in lamb 616 of the group given 200,000 larvae. The lowest hematocrit level was recorded the seventh week after the initial infection, and by the ninth week the hematocrit had risen to 30% and there was no subsequent decrease. The other member of the pair, lamb 617, maintained normal hematocrit levels during the same period. Lamb 616 also had the greatest decrease in hemoglobin, 3.1 g per 100 ml of blood. The other infected lambs displayed a decrease in hemoglobin of 0.6 to 2.7 g per 100 ml of blood. Thus, the hematocrit and hemoglobin values of the principals, except that of lamb 616, remained within normal limits throughout the experiment and were comparable to the uninfected controls prior to challenge.

Anemia did not develop in the infected lambs after they were challenged with 300,000 larvae, although a slight decline in the hematocrit reading from 29% to 27% occurred in lamb 617. However, lamb 649, which served as the challenge-control lamb, developed a moderate anemia after exposure to 300,000 larvae. The hematocrit declined from 34.5% to 26% and the hemoglobin from 10.8 to 8.2 g per 100 ml of blood in the 5-week period between infection and the termination of the experiment. The hematocrit and hemoglobin levels of the uninfected control, lamb 648, increased slightly during the same period.

None of the lambs in this experiment died, and none of the lambs receiving multiple doses, whether challenged or unchallenged, developed significant clinical symptoms, except the decreased rate of gain previously mentioned.

The lambs were killed 15 weeks after initial exposure to multiple infections, and 5 weeks after challenge. No pathology was noted in any of these animals. However, challenge-control lamb 649, which was given a single exposure of 300,000 larvae 5 weeks previous to autopsy, showed a generalized catarrhal enteritis throughout the duodenum and jejunum accompanied by erosion of the mucosa. This finding was similar to that previously observed in lambs subjected to single, massive invasions of larvae (Turner, 1955, 1959).

The numbers of worms, and percentages of larvae administered, which were recovered from the challenged and unchallenged lambs of each pair at autopsy were not large and only moderately higher in lamb 617, 1 of the 3 challenged lambs, than

in the unchallenged ones (Table I). These tabulated findings illustrate the immunizing action of the primary exposures to larvae.

Continuous Infections of Lambs and Kids on Pasture.

A slight retardation in weight gained by the principals as compared to the controls during their 9 weeks on pasture is shown in Figure 2. Infected lambs 613

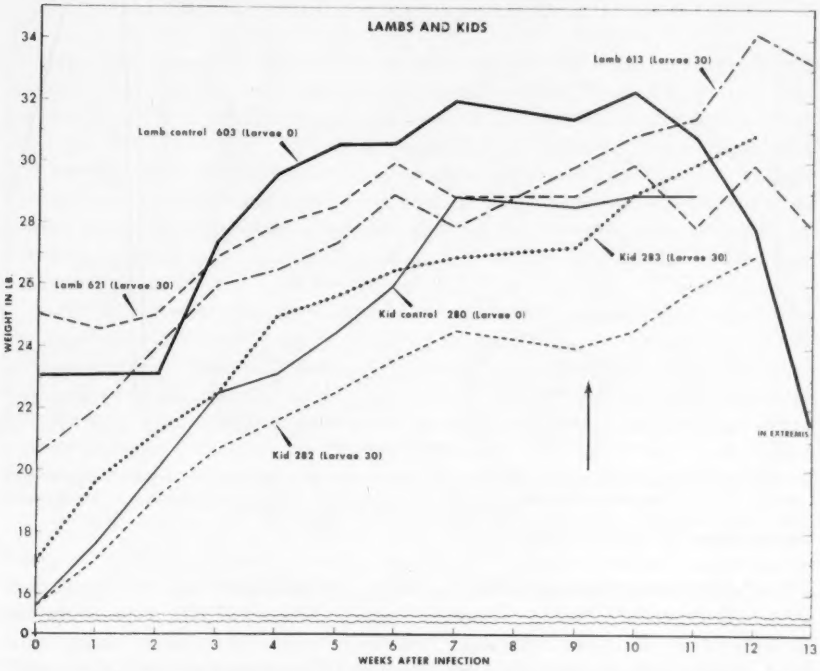

FIGURE 2. Weight data of individual lambs and kids infected on pasture. Arrow indicates when initial infection was challenged and controls were infected. Challenge-control kid 280 died 14 days after infection, and challenge-control lamb 603 killed in extremis 26 days after infection. Larvae in thousands.

and 621 gained 9½ pounds and 4 pounds, respectively, whereas the uninfected control lamb gained 8½ pounds. Infected kids 282 and 283 gained 9 and 10½ pounds, respectively, compared with 13½ pounds gained by the uninfected control. However, this trend was changed after challenge of the infected animals and initial exposure of the controls to 300,000 larvae. Four weeks after challenge, susceptible control lamb 603 had lost 10 pounds, whereas resistant lamb 621 lost 1 pound, and resistant lamb 613 gained 3 pounds. Susceptible kid 280 died 14 days after infection before any significant decrease in weight occurred, but it gained only ½ pound during this period in comparison to the 3 pounds gained by each of resistant kids 282 and 283.

Eggs of *S. papillosus* first appeared in the feces of all principals 9 days after infection (Fig. 3). During the prechallenge period of approximately 9 weeks, the

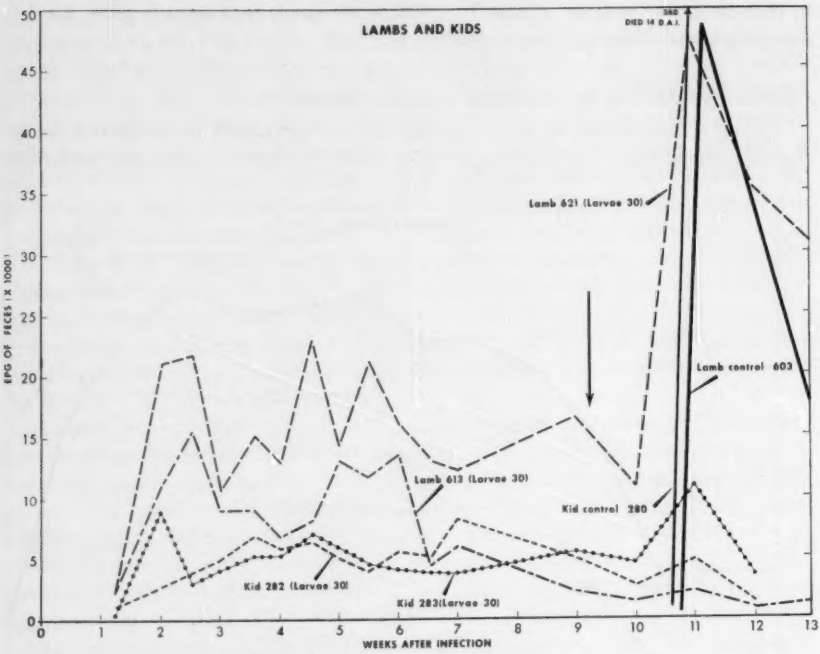

FIGURE 3. Worm egg counts of individual lambs and kids infected on pasture. Egg counts recorded for challenge-control lamb and kid during second week after challenge. Arrow indicates when animals receiving initial infection were challenged and controls infected with 300,000 larvae. Larvae in thousands.

lambs passed more worm eggs than the kids. The maximum numbers of eggs per gram of feces passed by lambs 613 and 621 were 15,750 and 23,100, respectively, and by kids 282 and 283 were 8,500 and 9,600, respectively. These peaks occurred 18, 32, 49, and 14 days, respectively, after infection. The number of worm eggs passed by these two lambs were relatively comparable to those of the lambs receiving 100,000 larvae, whereas those of the kids were roughly half this number. These maximum egg counts also were scattered over a period of 35 days, whereas those of the experimentally infected animals occurred over a period of 4 days.

Hematocrit percentages and hemoglobin levels decreased moderately in the infected lambs and kids during the first 9 weeks after the initial infection, and to a lesser extent or not at all in the uninfected controls. The hematocrit of lamb 613 decreased from 39 to 30% and of lamb 621, from 37 to 29%, whereas that of the uninfected control remained at 33%. The hematocrit of kid 282 decreased from 30 to 22%, that of kid 283 from 31 to 25%, and that of the control from 29 to 24%. The hemoglobin levels decreased 1.5 g per 100 ml of blood in lamb 613 and 2.1 g in lamb 621, whereas no significant change occurred in the control lamb. Greater decreases of hemoglobin occurred in the kids—3.7 g in kid 282, 2.6 g in kid 283, and 1.3 g in control kid 280.

At the time of challenge, 65 days after infection, the number of eggs of *S. papillosus* per gram of feces passed by the lambs and kids on pasture were as follows:

lamb 613, 2,280; lamb 621, 16,350; kid 282, 4,830; and kid 283, 6,330. Twelve days after challenge this number had increased significantly to 47,520 in lamb 621 and to 11,310 in kid 283. A week later the number of worm eggs had decreased below that recorded at time of challenge except in lamb 621, which maintained a high egg count until the experiment was terminated 4 weeks after challenge. The number of eggs per gram of feces passed by challenge-control lamb 603 attained a maximum of 48,540 12 days after initial exposure to 300,000 larvae and that of challenge-control kid 280 reached a maximum of 360,000 at the time of death (Fig. 3).

After the infected animals were challenged with 300,000 larvae, their hematocrit and hemoglobin levels did not decrease significantly except those of lamb 621. The hematocrit of this animal decreased from 29% to 24% and the hemoglobin declined 2.2 g per 100 ml of blood. In contrast, a moderate anemia developed in lamb 603 and kid 280, which served as challenge controls. The hematocrit of lamb 603 decreased from 33 to 24% and its hemoglobin declined 2.6 g per 100 ml of blood in the 4-week period subsequent to infection. During the 2-week period that kid 280 survived, its hematocrit declined from 24 to 16% and its hemoglobin level declined 4 g per 100 ml of blood.

Although various precautions were taken to exclude extraneous parasites from the experimental animals, various species of coccidia (*Eimeria intricata, E. ninae-kohl-yakimovi, E. arloingi,* and *E. faurei*) were found in all the lambs at various times after the 25th day on pasture. The kids apparently did not develop coccidial infections during their stay on pasture as determined by fecal examinations. Small numbers of eggs of *Haemonchus contortus* appeared in the feces of the lambs 63 days after they were placed on pasture, and a few eggs of *Moniezia expansa* were observed in fecal examination of lamb 621 late in the experiment.

Kid 280 died 14 days after exposure to 300,000 larvae. It was lethargic several days prior to death. Autopsy revealed peripheral congestion similar to pneumonitis in the lungs, fluid in the pleural cavity, ascites, and an enlarged gall bladder. The abomasum contained little food. Enteritis associated with thickened edematous areas confined to the duodenum was observed in the small intestine. Approximately 69%, or 205,850 threadworms, of the original dosage were recovered from this animal (Table II, K280). Of these, 4,500 were found in the abomasum; the rest in the small intestine. Many of these worms were in the fourth stage of development. The 2 challenged kids 282 and 283 were killed 20 days after challenge and a week after the challenge-control kid died. The organs of kid 282 revealed no gross pathology, and only 4,400 worms were recovered from the small intestine. In kid 283 there were a few petechiae in the upper diaphragmatic lobe of the left lung, possibly indicating recent passage of larvae through this region. The other organs appeared normal. A total of 17,700 worms were recovered, or approximately 4 times the number harbored by kid 282.

All the infected lambs (Table II) were killed on the same day, 26 days after challenge in the case of the principals (613, 621) and 26 days after infection in the case of the challenge control (603).

Autopsy revealed no gross pathology in lamb 613, and the carcass was in good condition. Only 2,900 *S. papillosus*, or less than 1% of the original infection, were recovered from the small intestine, and 6 *H. contortus* from the abomasum. Lamb 621 had ascites; otherwise, the findings at autopsy were normal. Approximately

13,608 *S. papillosus* and 70 *H. contortus* were recovered from this animal. Lamb 603 was moribund when it was killed. The necropsy revealed an enteritis, thickened duodenal mucosa, and fluid intestinal contents. All organs except the small intestine appeared to be normal. An estimated 82,338 worms were recovered from this animal. Although all animals were infected with various species of *Eimeria*, no coccidial lesions were noted in the intestines at autopsy. One tapeworm scolex was recovered from lamb 621.

DISCUSSION

These experiments show that a strong resistance to further infection with *S. papillosus* developed in the majority of the lambs exposed to immunizing infections in pens for a period of 20 days as well as in lambs and kids grazing for a period of about 2 months on a pasture that had been contaminated with the eggs and larvae of this parasite.

It is well known, however, that animals show considerable variation in the development of resistance to helminthic infection. Certain exceptions were also noted in these studies. Lamb 617, which was exposed to a total of 200,000 infective larvae given in multiple doses over a 20-day period, experienced a moderate exacerbation of infection when challenged, as determined by the number of worm eggs passed per gram of feces. However, some degree of resistance was established in this lamb, as only 8,028 worms (1.61% of the larvae administered) were recovered from it at autopsy, whereas 32,940 worms (10.98% of the larvae administered) were recovered from its challenge control. Another animal which apparently did not develop marked resistance to the same degree as the others was lamb 621 of the pasture experiment. This lamb after it was challenged passed a maximum of 47,520 EPG, whereas its uninfected control passed 48,540 EPG after receiving the single challenge exposure. The worms recovered from these two lambs, however, represented 4.1% and 27.5% of the number of larvae administered, respectively.

The data on the number of worm eggs passed, the changes in red cell volume, in the quantity of hemoglobin per 100 ml, and in body weight, as well as the autopsy findings, demonstrated that the immunizing infections greatly minimized the effects of a second or challenge exposure of lambs and kids to large numbers of infective larvae of *S. papillosus*. It appears significant that of the three susceptible challenge-control animals on pasture two died and one was severely affected. The reason that challenge-control 649 did not succumb to a number of larvae previously shown to be lethal is conjectural. It may be ascribed to individual variation in susceptibility or, perhaps, all of the larvae did not enter the skin. Nevertheless, the worm count at post mortem showed this animal to be more susceptible to the infection than the previously infected lambs.

These data support the results of certain other workers showing that an effective and persistent immunity develops in various hosts as a result of infection with other species of the genus *Strongyloides*. The development of resistance to reinfection with *S. stercoralis* in dogs has been reported by Sandground (1928), with *S. ransomi* in pigs by Enigk (1952a), and with *S. ratti* in rats by Sheldon (1937b). Chandler (1947) believed that helminths in the lumen of the intestine which feed on local tissues, such as *Strongyloides* spp., produce a localized, rapidly developing, powerful immunity. The development of such a localized immunity in the small intestine by repeated exposures to small numbers of larvae may explain why lambs were pro-

tected against massive challenge doses of infective larvae of *S. papillosus*. It seems probable that the larvae constituting the initial doses upon reaching the intestine and developing to maturity there excited an antigen-antibody reaction in the intestinal tissues, the antigen probably being some secretion or excretion of the parasite. The immunity mechanism of *S. papillosus* may be similar to the "self-cure" phenomenon seen in sheep infected with various trichostrongylid nematodes, as noted by Stewart (1955). These nematodes live in the abomasum and intestine of sheep, and heavily infected animals will lose almost the entire population of adult worms if large numbers of infective larvae of the same, or closely related, species are ingested. New infections of some species, however, according to Cameron (1956), may at times develop normally in sheep and cause fatalities. "Self-cure" appears to be due to a local abomasal or intestinal hypersensitivity caused by previous infections, which makes these habitats untenable for the adult parasites. This parallels the statement of Chandler (1953) that the intestinal immunization may be stimulated by older larvae or adult worms in the intestine. He also stated that in the case of nematodes which employ a parenteral phase of migration through the body, such as *Strongyloides* and *Nippostrongylus*, immunization may also be of the parenteral type and enhance intestinal resistance to the worms.

That *S. papillosus* can, under certain circumstances, cause a dermatitis in lambs was also demonstrated in the foregoing experiments. The erythema, urticaria, and increased sensitivity to touch that developed in the skin of the inguinal region of lambs after repeated applications of the larvae were similar to skin reactions of calves reported by Vegors (1954), and of rams and steers reported by Woodhouse (1948), which resulted from repeated cutaneous applications of larvae of this parasite. These authors reported a more intense reaction in cattle and sheep than that observed by the writer, which was probably due to the fact that they employed more larvae than the writer. These findings also paralleled those reported by Craig and Faust (1951) and Anon. (1955) on *S. stercoralis* in man and by Enigk (1952b) on *S. ransomi* of pigs, but were contrary to the observations of Sandground (1928), who stated that such sequelae were uncommon in dogs and cats reinfected with *S. stercoralis*.

SUMMARY

Immunizing infections against *S. papillosus* were induced in parasite-free lambs in isolation pens by cutaneous application of 10,000, 20,000, or 30,000 larvae at 2-day intervals for 20 days. Also, lambs and kids were initially infected by cutaneous exposures of 30,000 larvae each, and then grazed on a pasture where they were subsequently exposed to larvae of *S. papillosus* developing from eggs in feces spread on the area as well as from those in their droppings. None of these animals developed acute symptoms of strongyloidiasis. A strong resistance to further infection was demonstrated several weeks later by challenging one-half of the infected animals in pens and all on pasture with single, cutaneous exposures of 300,000 larvae, normally lethal for susceptible lambs and kids. Although the previously infected animals showed little effect of the challenge infection, 2 of the 3 parasite-free controls also exposed to 300,000 larvae at the time of challenge died.

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STUDIES ON TRICHINIASIS IN IOWA WILDLIFE (1955–56 AND 1956–57 SEASONS)*

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The significance of feeding unprocessed garbage to swine and the rat-swine relation in the transmission and spread of trichiniasis have been recognized for a number of years. Comparatively little consideration has been given to wildlife as a possible reservoir for trichiniasis infection. In recent years it has been found that various forms of wildlife act as reservoirs of infection for a number of diseases including parasitic conditions in man and domesticated animals. The Second National Conference on Trichinosis in 1954 recommended that wherever possible, species of wildlife should be examined to determine the prevalence of *T. spiralis*.

During the periods of 1944–46 and 1953 to date, a series of investigations has been carried out to determine the prevalence of *T. spiralis* in pok sausage and in Iowa swine, wildlife, dogs and cats, and to determine the possible inter-relations between trichiniasis in the various hosts encountered.

Zimmermann, Schwarte, and Biester (1956) recorded the prevalence of *T. spiralis* in wildlife for the 1953–55 interval as follows: rats (11.3%), mink (14.1%), adult foxes (11.9%), cub foxes (1.8%), opossum (2.5%), raccoon (0.9%), and coyote (50.0%). These appear to have been the first reports of trichiniasis in wild mink and the opossum.

MATERIALS AND METHODS

During the 1955–56 and 1956–57 hunting and trapping seasons, 1761 wildlife specimens were examined for *T. spiralis*. These specimens were obtained primarily from 10 sampling areas (Figure 1) but a number of others were obtained from the State Conservation Commission of Iowa. Animals examined included the following: mink, fox, opossum, raccoon, striped skunk, spotted skunk, coyote, and beaver, with other species being secured when available.

Samples of 45 g (0.1-pound) were examined by the artificial digestion-Baermann method. Tissues selected for examination were the diaphragm, tongue and leg muscle.

RESULTS

Mink (*Mustela vison*)

During the 1955–56 and 1956–57 seasons, 12 of 267 wild mink were found positive for *T. spiralis*, an overall prevalence of 4.5%. During the 1955–56 series, 6.6% of the mink were positive, while only 3.4% were infected in 1956–57. These figures indicate definite decreases over those reported from earlier studies, 17.9% being infected in the 1953–54 season, while in 1954–55 the prevalence was 11.1%. During 1955–56 the infected mink came from 2 of the 10 sampling areas, while in 1956–57

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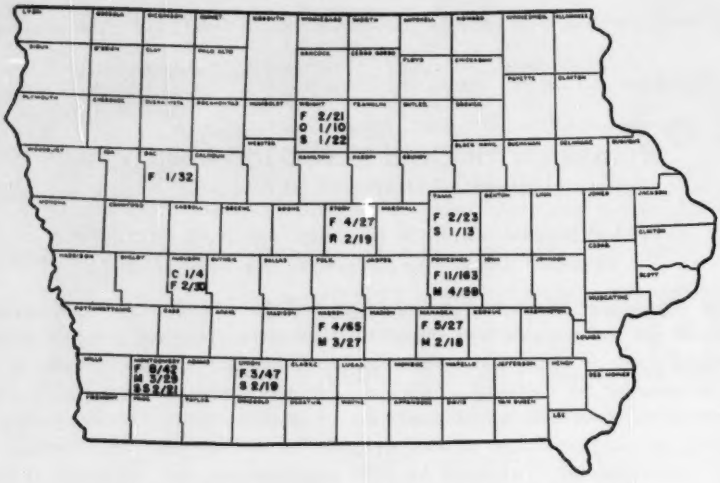

FIGURE 1: Sampling areas with distribution of infected animals*

* Code. C—Coyote; F—Fox; M—Mink; O—Opossum; R—Raccoon; S- Striped Skunk; SS—Spotted Skunk. First number signifies number of infected animals, second total number of that species examined.

infected mink were obtained from 3 areas. The intensity of the infections varied greatly. The maximum larval count obtained from a 45-g sample was 155,000, with 3 others containing more than 10,000. Five of the infections were light, larval counts being less than 120. Thirty-seven ranch mink were examined and found to be free from *T. spiralis.*

Red Fox (*Vulpes fulva*) and Grey Fox (*Urocyon cinereoargenteus*)

Most of the foxes examined were red foxes, only a few grey foxes being included. No attempt was made to differentiate between genera in these studies. During the two seasons 496 foxes were examined; 42 (8.5%) were found infected. *Trichinella* were isolated from 9.6% of the foxes during 1955–56, while the prevalence in 1956–57 was 7.6%. The previous prevalences found were 19.2% in 1953–54 and 7.6% in 1954–55. Twelve of the 21 infected foxes obtained during 1955–56 had larval counts in excess of 100, three exceeding 1000. The maximum count recorded was 1800. During 1956–57 only 6 of 21 counts were greater than 100, the maximum being 300. Five fox cubs were examined and found to be free of *T. spiralis.*

Striped Skunk (*Mephitis mephitis*)

The first striped skunk to be found infected with *T. spiralis* in Iowa was during the 1955–56 interval, when 1 of 24 striped skunks examined was positive. A 45-g sample from this striped skunk contained 4480 *Trichinella* larvae. In the 1956–57 season, 3 of 138 animals were positive. The 3 infected animals found during the 1956–57 season came from 2 areas. Larval counts of 1, 5, and 73 were obtained from the 45-g samples. The overall prevalence for the 2 seasons was 2.5%.

Spotted Skunk (*Spilogale interrupta*)

Two of 92 spotted skunks examined during these studies were infected with *T. spiralis* with samples containing 30,375 and 4455 trichinae, respectively. This is apparently the first report of trichiniasis in spotted skunks, more commonly but improperly known as civet cats in the Midwest. Both infected animals were obtained from the same area of western Iowa in the 1956–57 season.

Opossum (*Didelphis marsupialis*)

During the 2 seasons, 112 opossum were examined, one being positive. The infection was light, only 1 larva being found in the 45-g sample.

Raccoon (*Procyon lotor*)

The prevalence of trichiniasis continued at a low level in raccoons. Only 2 of 355 raccoons examined during the 2 seasons were found to be infected with *T. spiralis*. Both infected animals came from 1 area. The infections were light, with 45-g samples containing 45 and 56 trichinae, respectively.

Coyote (*Canis latrans*)

One of 21 coyotes examined during the 2 periods of the study was positive. This animal, obtained from southeastern Iowa, had 14 larvae in the 45-g sample.

Other species

In addition to the species of animals in which positive specimens were found, 220 other wild animals and birds were examined and were found to be free of *T. spiralis* infection. The following were included: badger, 8; bats, 4; beaver, 104; bob cat, 1; deer, 5; eagle, 1; fox squirrel, 1; hawks, 41; owls, 48; porcupine, 1; prairie dog, 1; weasel, 2; wolf, 1; woodchuck, 2.

Distribution

An examination of incidence by area (Fig. 1) shows that all 10 sampling areas had at least 1 species of wildlife infected with *Trichinella*. At least 1 infected fox was secured from each area. Nine of the 10 areas contained at least 2 positive species while 2 areas had 3. One area in southwestern Iowa had a prevalence of 10.3% in mink, 19.1% in foxes, and 9.5% in spotted skunks. The maximum prevalence found in any area for the various animals was mink, 11.1%; fox, 19.1%; striped skunk, 10.5%; spotted skunk, 9.5%; opossum, 10.0%; raccoon, 10.5%; and coyote, 25.0%.

DISCUSSION

The variety of hosts in which *Trichinella spiralis* has been found indicates that there are significant reservoirs of this parasite in the wild mammals of Iowa. Infections of various intensities have been found in 7 species of wild mammals in addition to rats. These species are foxes, mink, opossums, raccoons, striped skunks, spotted skunks, and coyotes. The finding of at least 2 infected species in 9 of the 10 sampling areas indicates that trichiniasis is not a localized problem, but must be considered in any area harboring wild animals.

Two modes of transmission of trichiniasis from wild animals to pigs must be considered in assaying the importance of the sylvatic occurrence of *T. spiralis*. These are (1) direct consumption of infected carcasses by swine, and (2) fecal

transmission by infected animals. Investigations (to be published) into these methods of transmission have shown that both may play important roles in the perpetuation of trichiniasis in grain-fed swine.

The possibility of fecal transmission of *T. spiralis* would emphasize the importance of foxes as potential vectors of infection to swine since they are relatively abundant in all parts of Iowa. They are nocturnal wanderers, sometimes being seen in hog lots. About 9% of the foxes examined have been infected with *T. spiralis*. Infected foxes have been obtained from all 10 areas of Iowa which have been checked.

The various species of wildlife other than the fox are probably of less importance in the transmission of trichiniasis to swine, even though they are quite common in Iowa. All of these animals are occasionally found in farm yards and adjacent fields. They enter hog lots and come in contact with many farm animals, but the relatively low prevalence of trichiniasis in these wildlife species would indicate that they cannot be considered a major source of infection to swine. If the relatively high prevalence of trichiniasis found in mink in the earlier studies had been maintained, the mink would have been considered an important potential vector, but the 4.5% incidence found during the later period diminishes their significance materially.

Notwithstanding the low prevalences of 0.9% found in opossums and 0.6% in raccoons, they might be considered as possible sources of trichiniasis in man. Opossums and raccoons are utilized as food by man in some areas, but generally the meat is well cooked before human consumption. The other 5 species of animals in which infections have been reported in this study would not commonly be used as human food.

The mammals in which *T. spiralis* has been reported are opportunists in their food habits. The fox and coyote persist mainly on meat diets, but fruits and other items may be consumed. The diet of the other 5 species consists of berries, fruits, insects, and other animals. Since small rodents may be an important source of trichiniasis in these animals, studies are now being carried out to determine the prevalence of *T. spiralis* in mice and other small rodents.

SUMMARY

Studies were conducted during the hunting and trapping seasons of 1955–56 and 1956–57 to determine the prevalence of trichiniasis in the wildlife of Iowa. *T. spiralis* has been found in 7 species of wildlife, namely mink (4.5%), fox (8.5%), opossum (0.9%), raccoon (0.6%), striped skunk (2.5%), spotted skunk (2.2%), and coyote (4.8%). This is apparently the first report of trichiniasis in the spotted skunk. Two or more infected species have been found from 9 of the 10 sampling areas.

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STUDIES ON *CLONORCHIS SINENSIS*. IV. PRODUCTION OF EGGS IN EXPERIMENTALLY INFECTED RABBITS*

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Observations on egg-production by *Clonorchis sinensis* in naturally and experimentally infected guinea pigs and cats by Faust and Khaw (1927) indicated that considerable variation existed between these hosts. The present investigation is concerned with the pattern of *Clonorchis* egg-production in a third host, the rabbit, over periods of up to 5 weeks after infection, together with observations on the daily output of eggs per worm.

METHODS

The sources of materials and the methods used in establishing experimental infections have been reported elsewhere (Wykoff and Lepeš, 1957; Wykoff, Part III, in press). A dilution egg-counting technic similar to that of Stoll's (1923) was used. One gram of fresh feces was weighed and diluted with 59 ml of N/10 NaOH. The following day the mixture was dispersed by shaking, the eggs counted in 0.1 ml of the suspension, and the number calculated to the basis of eggs per gram of feces. Prior to the 13th week of infection, when egg output was too limited for standard dilution egg-counting, a concentration technic was used to follow the initial increases.

Two groups of rabbits were used: (I)—7 given 300 metacercariae each, on which 4 or 5 replicate counts were made weekly or semi-weekly from the 13th week of infection to the time of death, or 55 weeks, whichever occurred later, and (II)—9 animals given from 50 to 1000 metacercariae, on which egg counts were made only during the 17th week of infection at the end of which time they were sacrificed. At the termination of the observation periods, the worms were removed from the liver and these numbers correlated with egg counts.

RESULTS

Egg-production increased evenly from the beginning of patency at the 22nd day to the 13th week after which there was cyclic fluctuation at roughly 10-week intervals. For each of the 7 animals in Group I, the variability between the 4 or 5 replicate counts was in close agreement with that expected for a Poisson variable (Fig. 1). The first peak of egg-production occurred between weeks 16 and 17 for 5 of the 7 animals; the others at weeks 13–14 and at week 19. At these times the egg-production ranged from 8,000 to 30,000 eggs per gram feces (EPGF) with a mean of 18,000. The lowest production of eggs following the first peak occurred during weeks 22–25 for the 6 surviving animals, the range of EPGF being 2,000 to 6,000 with a mean of 4,000. In all cases, second peaks were then evidenced at weeks 25 to 29 with counts averaging 17,000 EPGF. Subsequent minimal egg-production occurred during weeks 34–36 for the 4 surviving animals, with a mean of 4,000 EPGF. The final observed peaks of egg-production occurred from weeks 38 to 40

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FIGURE I

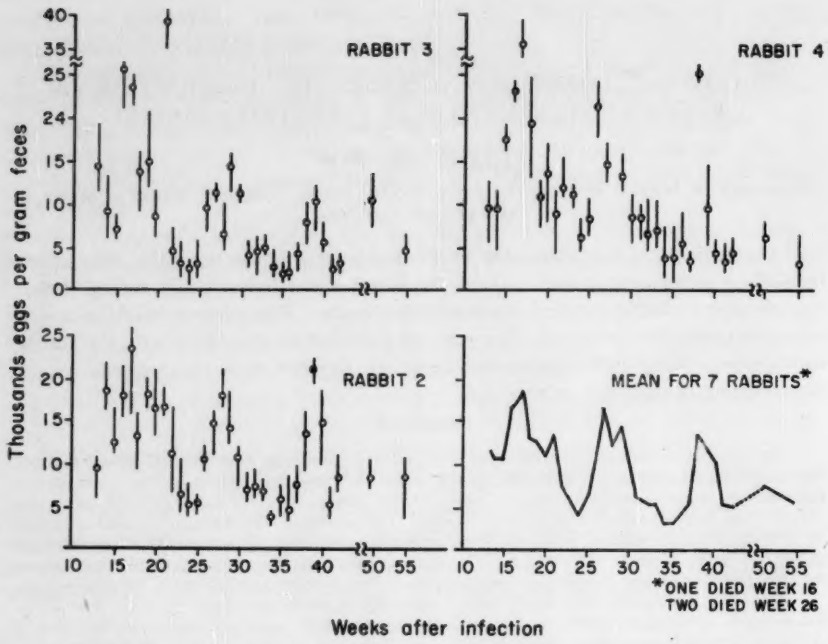

FIGURE 1. Range (|) and mean (○) of *Clonorchis sinensis* eggs per gram of feces based on 5 dilution counts made weekly on each of 3 rabbits selected as typical, and the combined mean for 7 such rabbits, for the period 13 to 55 weeks after inoculation.

with an average of 18,000 EPGF. The ranges and means of EPGF for the individuals and the averages for all 7 rabbits are in close agreement, all showing much the same trends and levels (Fig. 1, Table I).

TABLE I.—*Weeks after inoculation at which highest and lowest egg-production occurred and (in parentheses) thousands of eggs per gram of feces for* Clonorchis sinensis *in 7 rabbits.* (For graph of mean see Figure 1.)

Rabbit Number	First Period of Egg Output		Second Period of Egg Output		Third Period of Egg Output
	High	Low	High	Low	High
1	13–14 (20)	24–25 (4)	27 (32)	35 (4)	40 (16)
2	16–17 (21)	24–25 (5)	28 (18)	34–35 (5)	39 (20)
3	16–17 (25)	24–25 (3)	29 (14)	35–36 (2)	39 (10)
4	16–17 (30)	24 (6)	26 (21)	34–35 (5)	38 (26)
5*	16 (11)	—	—	—	—
6**	16 (12)	23–24 (2)	26 (10)	—	—
7**	19 (8)	22 (3)	25 (9)	—	—

* Animal died during week 16.
** Animal died during week 26.

The means of the egg counts made during the week prior to death or sacrifice (weeks 16 to 55 for Group I; week 17 for Group II) correlated with the numbers of worms recovered, showed ranges of 954 to 6,500 with a mean of $3,780 \pm 1,330$ (SE) eggs per worm per day, and 34 to 334 with a mean of 106 ± 72 (SE) eggs per worm per gram of feces (Table II).

TABLE II.—*Production of eggs by* Clonorchis sinensis *based on replicate dilution counts made during the week of death.*

Week of Death	Rabbit No.	Number of Worms Recovered	Average egg output per worm	
			Thousands/day	Eggs/gram feces
16	5	73	3.7*	149
17	8	8	5.3	75
17	9	35	5.6	73
17	10	11	2.7	54
17	11	41	4.1	121
17	12	58	2.4	34
17	13	53	2.6	45
17	14	24	4.1	63
17	15	454	3.4	70
17	16	301	3.8	70
26	7	72	4.1	121
26**	6	48	6.5	202
50**	1	42	2.5	97
55**	2	68	4.0	128
55**	3	14	4.5	334
55**	4	50	1.0	60
Mean			3.78	106
SE			1.33	72

* Estimated egg output based on four or five (**) replicate counts.

DISCUSSION

Previous studies have indicated that the prepatent period for *Clonorchis* in rabbits is approximately 22 days (Wykoff, Part III, in press). It appears that egg-production increases steadily and regularly from about the third week after inoculation and reaches a maximum at approximately 17 weeks. Thereafter, fluctuations seem to occur in cyclic fashion with peaks at approximately 10-week intervals. No explanation of this periodicity is known but several suppositions may be made. Certain metabolic functions of the fluke may cause cyclic egg-production, or perhaps egg-production is constant and the eggs are caught in the biliary crypts and liberated periodically. A third possibility may be related to host resistance to the parasite. However, complement-fixing antibody titers determined weekly on these animals (Wykoff, Part II, in press) had no apparent correlation with levels of egg-production.

Knowledge of the average output of eggs for *Clonorchis sinensis,* although only a general approximation, may permit rough estimations of the worm burden. However, it appears from the fluctuations observed in the present study that the duration of infection at the time of the counts is an important factor.

Faust and Khaw (1927) studied egg-production in 7 cats and 5 guinea pigs. Of the cats, 4 were natural infections of unknown duration and 3 were experimental infections. Of the latter, counts were started 9, 18, and 61 days after infection and were continued for 13, 13, and 26 days, respectively. The guinea pig infections were all experimental. Egg counts were started 13, 14, 16, 18, and 80 days after infection, and continued for 25, 26, 29, 22, and 37 days. Thus the range of times from infection to termination of counts was 22 to 87 days, with a mean of 47 days for the cats, and 38 to 117 days with a mean of 56 days for the guinea pigs. The fact that maximum egg-production in the present work did not occur before some 119 days after infection in rabbits is suggestive that a similar period may be required in cats and guinea pigs. If such is the case, Faust and Khaw's findings on cats (2,400 eggs per worm per day), and guinea pigs (1,600 eggs per worm per day) may have been somewhat higher if the counts had been made after complete maturing of the worms. However, the estimates for these animals are in general agreement with those on rabbits and it appears that a rough approximation of worm burden in such experimental animals may be made on the basis of the fecal egg counts.

SUMMARY

Egg-production by *Clonorchis sinensis* in 7 rabbits was followed by a dilution egg-counting technic for periods up to 55 weeks after inoculation of metacercariae. The numbers of eggs increased steadily up to the 17th week and thereafter showed cyclic variation with maxima at roughly 10-week intervals. The mean number of eggs per worm per day, based on counts made during the week of death in 16 animals, was approximately 4,000 and the mean number of eggs per gram of feces per worm was approximately 100.

ACKNOWLEDGMENTS

The author is indebted to Professor P. C. Beaver, Tulane University, for numerous suggestions, and to Mr. R. E. Duxbury, Walter Reed Army Institute of Research, for technical assistance.

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RESEARCH NOTE

SIMULTANEOUS INFECTIONS WITH *PLASMODIUM BERGHEI* AND *TRYPANOSOMA LEWISI* IN THE RAT

Simultaneous infections with *Plasmodium berghei* and *Tryanosoma lewisi* were followed in young (60–120 g) female Holtzman rats by daily blood films from the tail vein. The air dried films were fixed in methyl alcohol for 3 minutes and stained for 25 minutes with stock Giemsa's stain diluted 1 : 20 in pH 6.8 buffer.

Of 33 rats inoculated intraperitoneally with citrated blood containing large numbers of *P. berghei* and *T. lewisi*, 25 became infected with both parasites and 8 with only *T. lewisi*. The two parasites did not significantly alter each other's course of infection and increases in numbers of parasites and crises were comparable to single infections.

Single infections with either parasite are seldom fatal for this host but 17 of the 25 rats with simultaneous infections died; this may be ascribed to a very severe anemia. The peripheral blood was depleted of mature erythrocytes and reticulocytes to such an extent that nucleated erythrocyte precursors appeared in the circulation. *P. berghei* preferentially invades reticulocytes but also parasitizes mature erythrocytes and the nucleated precursors.

This study was supported by U. S. Public Health Service Grant No. E-884(C3).—GEORGE J. JACKSON, *Department of Microbiology, The University of Chicago.*

TREMATODES OF GHANAIAN SUB-LITTORAL FISHES. I. THE FAMILY MONORCHIIDAE

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The trematodes of the family Monorchiidae described in the present paper form part of a collection of parasitic worms obtained from marine fishes. The fishes were captured in the sub-littoral zone off the coast of Ghana from December 1954 to April 1956.

There are numerous descriptions and records of the trematode parasites of marine fishes from various parts of the world, the majority of which have been conveniently summarized and reviewed by Dawes (1946, 1947), Yamaguti (1953), Skrjabin (1947–1955), and Manter (1955). A consideration of these reviews shows that the West African coast has received little study. With the exception of the work of Dollfus (1937) there appear to be no records of trematodes of fishes from this area. The present work was undertaken with the view to furthering knowledge of the taxonomy, geographical distribution and specificity of the trematodes of marine fishes.

MATERIALS AND METHODS

The marine fishes examined for parasites were captured by the Government Fishery Department and by the Government Prison Fisheries in the vicinity of Sekondi and Accra respectively. (Fig. 1.) Fishes obtained from the Prison fisheries were captured by means of large seine nets

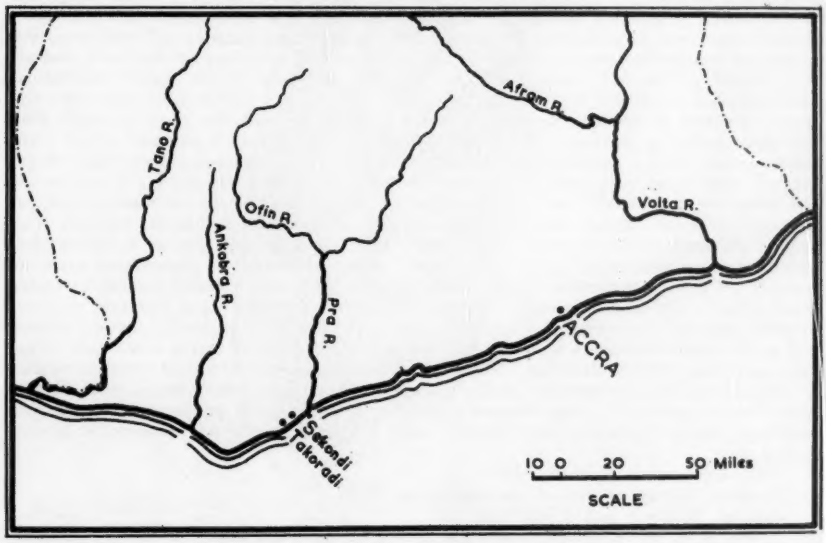

FIGURE 1. Map of the coastline investigated, showing the position of the sampling stations.

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fished from the shore and those from the Government fisheries by trawlers fishing within a mile or 2 from the shore. In both cases, therefore, only fishes inhabiting the sub-littoral fringe were captured. The fishes were examined for parasites as soon after capture as possible. At the commencement of the work specimens of parasites were fixed under slight pressure from a coverglass in Gilson's fluid but subsequently they were fixed in 5% formalin or 75% alcohol as recommended by Johri and Smyth (1956). All measurements, therefore, apply to slightly flattened specimens and are given below in mm. Whole mounts were stained with a variety of stains including Ehrlich's haematoxylin, Caruzzi's haematoxylin, Mayer's paracarmine and Gower's carmine. Transverse serial sections 6 microns in thickness were prepared in the case of one species and stained in Ehrlich's haematoxylin and Orange G. The species encountered are considered in turn below.

Lasiotocus cynoglossi n. sp.

Material of this species was obtained from the intestine of the tongue-sole, *Cynoglossus goreensis* Steind. from both collecting areas. The populations of this species from the 2 areas show certain interesting differences which are deemed subspecific in extent and are described below.

a. *L. cynoglossi magniovatus* n. sp., n. sub. sp. from the Accra area (Figs. 2–3)

Diagnosis: Body elongated; with more or less rounded ends; 1.0 to 1.54 long; 0.28 to 0.34 broad at widest point which is in the testicular region. Body surface covered with small backwardly directed spines which become progressively less dense posteriorly. Oral sucker subterminal; almost spherical; 0.09 to 0.15 in diameter. Ventral sucker in anterior third of body; separated from oral sucker by a distance ranging from 0.08 to 0.23, depending on degree of contraction of specimen; almost spherical; 0.12 to 0.20 in diameter; ratio of oral to ventral sucker 1 : 1.30 to 1 : 1.41. Extrinsic muscles poorly developed. Oral opening antero-ventrally situated on oral sucker, leading into a short prepharynx. Pharynx oval or spherical; 0.04 to 0.05 long; 0.05 to 0.07 broad. Esophagus short; up to 0.03 long; leading into postero-laterally directed gut-ceca which terminate a short distance from posterior extremity. Excretory pore subterminal at posterior end, opening into excretory bladder which extends anteriorly in median dorsal field to level of vitelline reservoir before bifurcating to give rise to antero-laterally directed main excretory ducts. Genital atrium elongated, lateral to ventral sucker or overlapping it; extending from posterior margin of ventral sucker to genital pore. Male and female ducts may open close together at base of atrium (Fig. 2) or cirrus may be everted and female terminal organ protruded into anterior portion of atrium as shown in case of *L. cynoglossi major* from Sekondi area (Fig. 4). Testis single; compact or faintly lobed; longitudinally oval; 0.12 to 0.21 long; 0.10 to 0.15 broad; situated in median line, a short distance behind ovary. Vas deferens short; arising from antero-dorsal margin of testis. Cirrus pouch large; arcuate; with well developed circular or longitudinal muscles; length variable; 0.25 to 0.37 depending on degree of contraction; breadth 0.09 to 0.11; basal portion with vesicula seminalis; middle region with tubular pars-prostatica of variable length; terminal region with cirrus. Cirrus beset with numerous spines; pyriform or tubular depending on degree of eversion; remainder of cirrus pouch filled with densely-staining prostatic cells. Ovary amphitypic, lying lateral to proximal end of cirrus pouch; oviduct short, arising dorsally from ovary and enlarging in the median line to form ootype; Laurer's canal short, originating from proximal region of oviduct and opening to exterior in mid-dorsal line above ootype; median yolk duct enters oviduct shortly before formation of ootype; receptaculum seminis absent; Mehlis's gland poorly developed. Uterus emerges ventrally from ootype; proximal region of uterus dilated with sperm and eggs and acts as a receptaculum seminis uterinus; descending and ascending limbs of uterus fill most of post-ovarian space not occupied by testis and vitelline glands; distal portion leads into a highly modified terminal organ which is surrounded on outside by glandular cells. Terminal organ bipartite; posterior chamber spherical, containing sperm; anterior chamber beset with bristle-like spines; opening into genital atrium through a muscular bulb-like sphincter; receiving uterus at its base. Eggs oval; operculate; 0.027 to 0.030 long, 0.02 broad. Vitelline glands consisting of numerous tubular or more rounded follicles, situated laterally beneath gut-ceca and extending from a short distance behind ventral sucker to a short distance in front of terminations of gut-ceca; transverse yolk duct large, situated in narrow space between ovary and testis; giving rise to ventrally directed median duct.

Host: Cynoglossus goreensis Steind.
Locality: Sub-littoral area of sea near Accra, Ghana.
Habitat: Posterior part of intestine.
Types: In the helminthological collection, Zoology Department, University College of Ghana.
No. of specimens: 12.

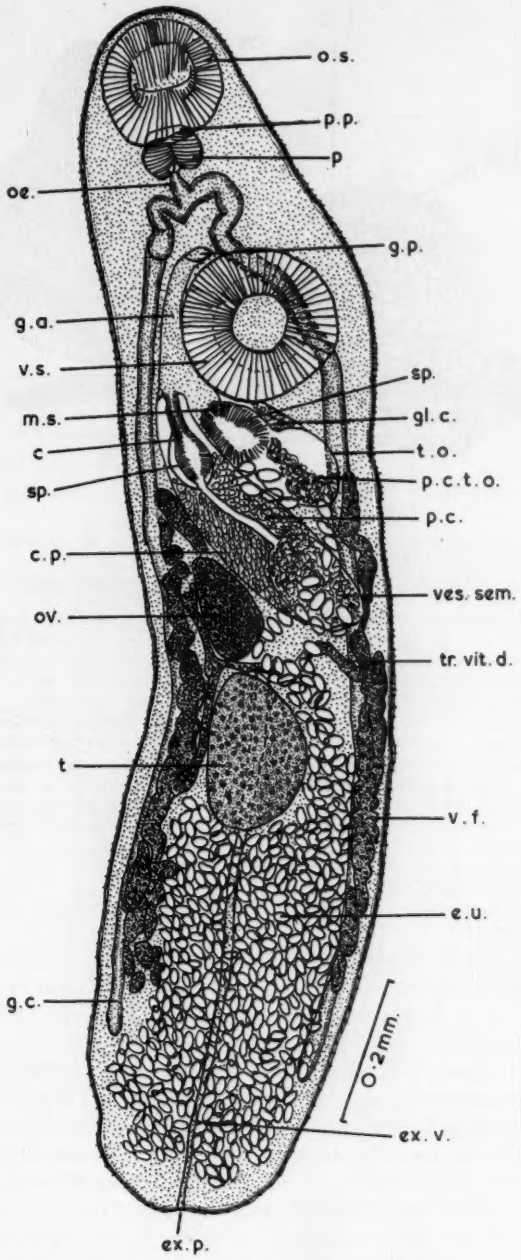

FIGURE 2. *Lasiotocus cynoglossi magniovatus* n. sp., n. sub. sp., ventral view.

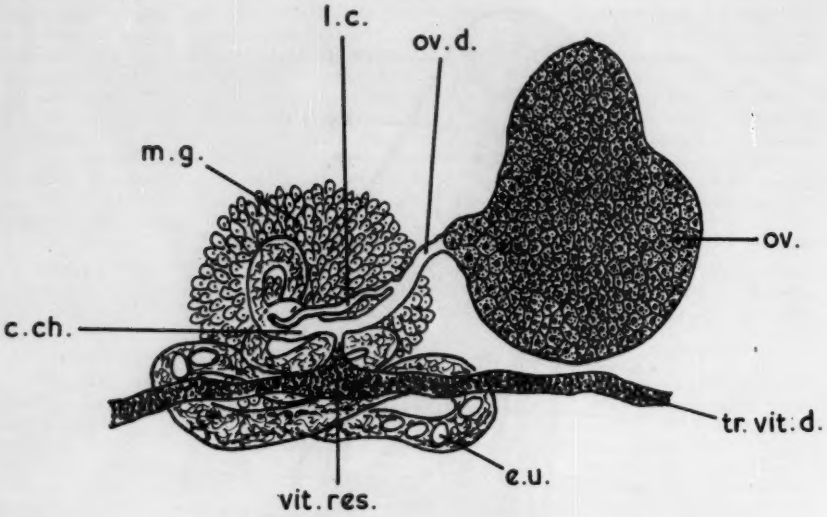

FIGURE 3. Ovarian complex of *L. cynoglossi magniovatus* n. sp., n. sub. sp.

b. *L. cynoglossi major* n. sp., n. sub-sp. from the Sekondi area (Fig. 4)

Diagnosis: Measurements based on 6 mature specimens are as follows: Length 0.81 to 4.01; breadth 0.55 to 1.03; oral sucker 0.10 to 0.25 by 0.11 to 0.25; ventral sucker 0.15 to 0.37 by 0.14 to 0.33; ratio of oral to ventral sucker 1 : 1.46 to 1 : 1.65; distance between suckers 0.16 to 0.80; pharynx 0.09 to 0.14 by 0.11 to 0.15; testis elongated transversely, 0.10 to 0.33 by 0.18 to 0.48; ovary 0.08 by 0.39 to 0.08 by 0.26; distance from ventral sucker to posterior 0.74 to 1.03; eggs 0.015 to 0.020 by 0.012 to 0.015; vitelline glands tubular often showing diffuse branching, rarely as compact as in forms from the Accra area, covering an area of from 0.08 by 0.25 to 0.36 by 2.04.

Certain of the differences between the trematodes from the 2 areas cannot be attributed to size differences. These include differences in the egg-size, the ratios of the oral and ventral sucker and the form of the testis and the vitelline glands. These differences which are discussed below are probably due to genetical factors but are considered to be, at most, sub-specific in extent.

Host: Cynoglossus goreensis Steind.

Locality: Sub-littoral area of sea near Sekondi, Ghana.

Habitat: Posterior region of intestine.

Type: In the helminthological collection, Zoology Department, University College of Ghana.

No. of specimens: 6.

The question of the synonymy of *Lasiotocus* Looss, 1907, *Genolopa* Linton, 1910, *Proctotrema* Odhner, 1911, *Paraproctotrema* Yamaguti, 1934 and *Proctotrematoides* Yamaguti, 1938 is a subject which has already occupied the attention of many authors. Lloyd and Guberlet (1932) and Yamaguti (1934) considered the probable synonym of *Proctotrema* Odhner, 1911 and *Genolopa* Linton, 1910 but were unable to reach a definite conclusion owing to lack of information. Manter (1940) on the other hand had suggested tentatively that *Genolopa ampullacea*, Linton's type species was generically distinct from *Proctotrema* on the basis of 2 kinds of spines associated with the cirrus sac. Later, Manter (1942) reaffirmed this conclusion although Hopkins (1941) had earlier claimed that the spination of the cirrus in *G. ampullacea* is in no way exceptional and that *Proctotrema* and *Genolopa* are congeneric. Manter admits, however, to having been in error in his earlier paper in describing the atrial spines as a median cluster of spines in the cirrus. Other suggestions put forward by Manter (1942) are that *Proctotrematoides* Yamaguti, 1938 should be regarded

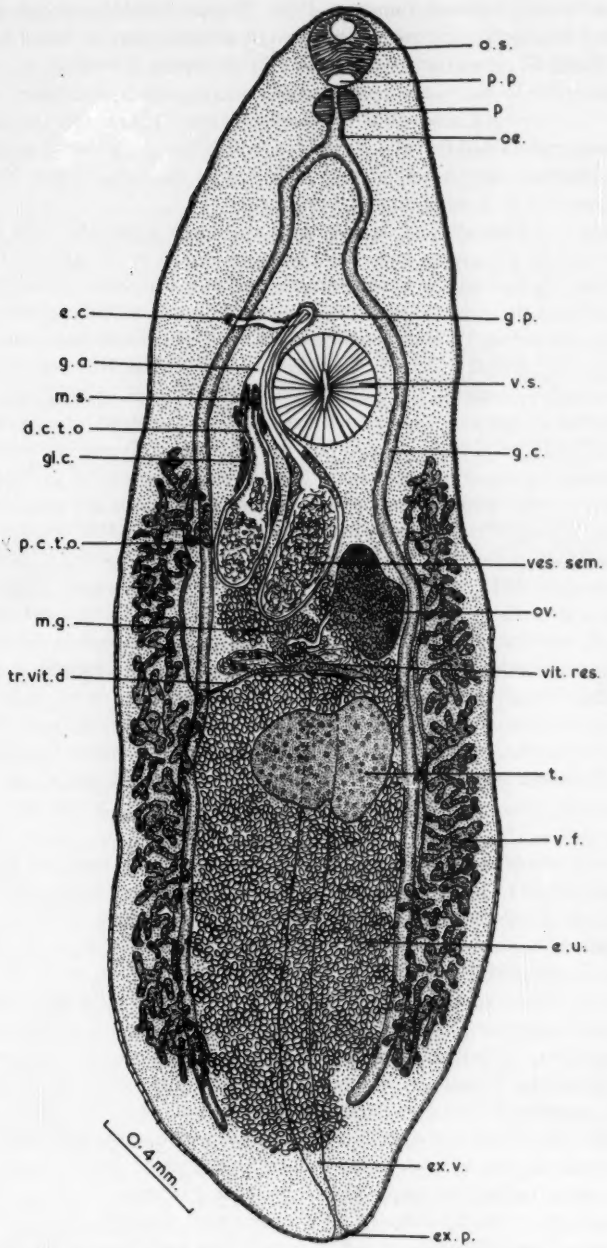

FIGURE 4. *Lasiotocus cynoglossi major* n. sp., n. sub. sp., dorsal view.

as a synonym of *Genolopa* and that *G. elongatum* Manter, 1931 should be transferred to the genus *Paraproctotrema* Yamaguti, 1934. Nagaty (1948) and Sobolec (1955) have accepted Manter's recommendations and in addition have included *G. trifolifer* Nicoll, 1915 and *G. cacuminata* Nicoll, 1915 in the genus *Genolopa*.

Yamaguti (1953) has more recently reduced *Proctotrema* Odhner, 1911 to a synonym of *Lasiotocus* Looss, 1907 although Odhner (1911) and Dollfus (1948) had previously maintained that the 2 genera are distinct. In a recent personal communication Dollfus states, however, that he is now of the opinion that *Proctotrema* should be regarded as a sub-genus of *Lasiotocus*.

According to Yamaguti (1953) *Genolopa* should be separated from *Lasiotocus* on the ground that the uterus enters the terminal organ at its apex in the former genus and near its base in the latter genus. The practical value of this distinction is doubtful as in many of the original descriptions the point of entry is not mentioned and in others including *G. plectorhynchi* Yamaguti, 1915; *G. cacuminata* Nicoll, 1915; *G. trifolifer* Nicoll, 1915; *G. longicaecum* Manter; and *L. himezi* Yamaguti it is roughly midway between the anterior and posterior ends of the terminal organ. The distribution of species proposed by Yamaguti is, therefore, unacceptable.

Manter (1942), as already pointed out, has offered an alternative way of distinguishing between the controversial genera *Genolopa* and *Lasiotocus* (=*Proctotrema*). On this basis several allied species might be included within the genus *Genolopa*. These are as follows: *Proctotrema plectorhynchi* Yamaguti, 1934, *Paraproctotrema brevicaecum* Manter 1942, *L. lethrini* Yamaguti, 1953, *Proctotrematoides pisodontophidis* Yamaguti, 1938, and *Paraproctotrema fusiforme* Yamaguti, 1934. Further examination shows that the spines figured in the atria of *P. brevicaecum* and *P. plectorhynchi* are not described in the text as atrial spines and can be interpreted as spines of the terminal male and female ducts. These 2 species cannot, therefore, be included within the genus *Genolopa*. *L. lethrini*, on the other hand, is described as possessing atrial spines and should be transferred to the genus *Genolopa*. In view of the fact that *P. pisodontophidis* differs fundamentally from *G. ampullacea* in possessing an atrial diverticle and an excretory vesicle of pre-acetabular extent it would seem correct to retain the genus *Proctotrematoides* as suggested by Yamaguti (1953).

The generic status of *Paraproctotrema* has been upheld by many authors including Yamaguti (1934), Manter (1942), Nagaty (1948) and Sobolec (1955) on the basis of the well developed sphincter surrounding the distal end of the uterus prior to the formation of the terminal organ and the presence of the spiny atrium. The inclusion of *G. cacuminata* Nicoll, 1915 and *G. elongata* Manter, 1931 by Yamaguti (1934) and *P. brevicaecum* by Manter (1942) in the genus *Paraproctotrema* appears unjustified, however, as none of these species possess a spiny atrium or the characteristic sphincter. A detailed consideration of the type species of *Paraproctotrema* namely *P. fusiforme* Yamaguti shows that except for the presence of the uterine sphincter it conforms to the generic diagnosis of *Genolopa*. This character would appear insufficient to justify the erection of a genus and it seems advisable to reduce *Paraproctotrema* to synonymy with *Genolopa* as suggested by Yamaguti (1953).

Three species, namely *G. ampullacea* Linton, *G. fusiforme* (Yamaguti) and *G. lethrini* (Yamaguti), can be included within the genus *Genolopa*. It seems best to retain the genus *Genolopa* unless it can be proved that the spines in the genital atria of these species are those of the everted cirrus or protruded female terminal organ.

With the exception of these 3 species and *Proctotrematoides pisodontophidis* Yamaguti, all the species previously allocated to the genera *Lasiotocus, Proctotrema, Paraproctotrema,* and *Proctotrematoides* appear to conform to the generic diagnosis of *Lasiotocus* as given by Looss (1907). The diagnosis given by Dollfus (1948) and Yamaguti (1953) must be emended to indicate the variability of certain features including the point of entry of the uterus into the terminal organ.

It is noteworthy, in this connection, that there is considerable variation in the descriptions of the excretory systems of species allocated to this genus indicating that they may form a heterogeneous assemblage but in view of the incompleteness of our knowledge concerning the excretory system in certain species further work is necessary before this character can be used for classification purposes.

Lasiotocus Looss, 1907. emend.

Generic diagnosis: Monorchiidae Odhner 1911. Body spinulate, more or less fusiform. Oral sucker almost spherical or funnel shaped. Prepharynx present. Pharynx large, oval or almost spherical. Oesophagus short. Ceca simple, terminating at posterior extremity or shortly behind level of testis. Acetabulum in middle third of body, larger or smaller than oral sucker. Testes single, preacetabular. Cirrus pouch with armed cirrus and vesicula seminalis, extending backward beyond acetabulum. Genital pore preacetabular. Genital atrium short or long. Ovary compact or lobed, sub-median, pretesticular. Receptaculum seminis rudimentary if present. Laurer's canal present. Vitellaria occur in bunches of follicles arranged laterally in acetabulotesticular region or extending almost beyond posterior region of gut ceca. Uterus occupying most of space in hind body, the proximal region acting as a well developed receptaculum seminis uterinus, terminal organ of uterus usually bipartite with or without a well developed sphincter anteriorly. Excretory vesicle short or extending to testicular region before bifurcating. Parasites in intestine of marine fishes.

Genotype *Lasiotocus mulli* Looss, 1907.

The species included in this genus in addition to the ones described in the present paper, are as follows: *L. bacilliovatus* (Odhner, 1911), *L. brevicaecum** (Manter, 1942), *L. beauforti** (Hopkins, 1941), *L. cacuminatus** (Nicoll, 1915), *L. costaricae* (Manter, 1940), *L. elongatus** (Manter, 1931), *L. himezi* Yamaguti, 1951, *L. lintoni** (Manter, 1931), *L. longicaecum* (Manter, 1940), *L. longovatus** (Hopkins, 1941), *L. macrorchis* (Yamaguti, 1934), *L. malasi* (Nagaty, 1948), *L. minutus** (Manter, 1931), *L. mulli* (Stoss., 1883) Looss, 1907, *L. odhneri* (Srivastava, 1939), *L. parvus* (Manter, 1942), *L. plectorhynchi* (Yamaguti, 1934), *L. trifolifer** (Nicoll, 1915) and *L. truncatus** (Linton, 1910).

L. cynoglossi differs from all other *Lasiotocus* species in possessing vitelline follicles extending from the acetabular level to the posterior end of the gut ceca and from all other species with the exception of *L. beauforti* in possessing an unusually long genital atrium. These differences are deemed insufficient to justify generic status.

There remain some doubts concerning the taxonomic position of certain species allocated to the genus *Lasiotocus.* Two species namely *L. trifolifer* and *L. cacuminata* may be described as *species inquirendae,* although they agree in the main with the generic diagnosis of *Lasiotocus.* It seems clear that they cannot be placed in the genus *Genolopa* in view of the fact that no spines are described from the genital atrium of either species. Certain other species appear not to be valid. Thus *L. longovatum* Hopkins is probably a synonym of *L. lintoni.* Nagaty (1948) also claims that *L. odhneri* is a synonym of *L. lintoni* and *L. beauforti* is a synonym of *L. longicaecum.* The question of the synonymy of the latter 2 species is discussed below.

* new combination

Lasiotocus longicaecum (Manter 1940) (Fig. 5)

Two specimens of *L. longicaecum* recovered from *Lethrinus atlanticus* C. & V. near Accra during the present investigation are almost identical with the 3 specimens described by Manter (1940) from *Anisotrema interruptus* (Gill) and *A. virginicus* (L.) found near the coast of Florida and the Galapagos Islands except for minor differences in the sizes of the oral suckers. In view of the unknown variability of this species these differences are considered inadequate to justify the erection of a new species.

Nagaty (1948) claims that *L. beauforti* is a synonym of *L. longicaecum* but as *L. longicaecum* has a much shorter genital atrium and a longer excretory bladder than *L. beauforti* this is considered unlikely. Unfortunately Manter's description of the excretory system and certain other anatomical features is incomplete. Consequently a more complete description based on present specimens is given below.

Diagnosis: Body oval or more elongated; extremities almost truncate; 1.4 to 1.7 long; 0.46 to 0.47 broad at widest point in region of vitelline glands. Body surface covered with small, backwardly directed spines. Oral sucker sub-terminal, funnel shaped, 0.21 to 0.23 by 0.17 to 0.20. Ventral sucker in anterior half of body, separated from oral sucker by distance varying from 0.25 to 0.32; almost spherical, 0.12 to 0.14 by 0.12, appreciably smaller than oral sucker. Ratio of oral to ventral sucker 1.50 : 1 to 1.75 : 1. Oral opening antero-ventral, opening into short prepharynx. Pharynx oval; 0.065 by 0.05 to 0.10 by 0.10. Esophagus short. Gut-ceca long; postero-laterally directed, terminating a short distance from posterior extremity. Excretory pore terminal, posteriorly. Excretory bladder tubular, reaching almost to ventral-sucker before bifurcating. Genital pore median, immediately preacetabular. Genital atrium short, oval. Testis single, median, with longitudinal axis diagonally placed; 0.23 to 0.24 by 0.12 to 0.15. Vas deferens short. Cirrus pouch large, up to 0.46 by 0.10, with spherical vesicula-seminalis, short tubular pars-prostatica, armed cirrus with large elongated spines, and prostatic cells. Ovary to left of mid-line, lateral to anterior margin of testis, compact, oval, 0.10 to 0.12 by 0.07 to 0.11. Ootype and Mehlis's gland median at ovarian level. Laurer's canal short, running antero-laterally from a slightly swollen portion of oviduct and opening dorsally near base of cirrus pouch; swollen portion of oviduct may represent vestigial receptaculum-seminis. Uterus emerges ventrally from ootype; proximal region acts as a receptaculum-seminis-uterinus; descending and ascending limbs of uterus fill most of post-ovarian space. Terminal organ of uterus well developed, consisting of proximal, almost spherical region with sperms and a terminal spined portion. Uterine spines approximately twice the length of cirrus spines. Uterus enters proximal region of distal chamber. Eggs oval; 0.015 to 0.020 by 0.0125 to 0.015. Vitelline follicles situated in 2 lateral groups at testiculo-ovarian level, overlapping gut-ceca. Transverse yolk duct at ovarian or pre-ovarian level giving rise to median, dorsally directed yolk duct.

Lasiotocus chaetodipteri n. sp. (Fig. 6)

Diagnosis: Body elongate oval; 0.80 to 1.07 long; 0.24 to 0.30 broad at widest point. Body surface covered with spines. Oral sucker funnel shaped, sub-terminal, 0.15 by 0.16 to 0.15 by 0.15. Ventral sucker in anterior third of body, almost circular, 0.08 to 0.09 by 0.08; appreciably smaller than ventral sucker. Ratio of oral to ventral sucker 1.66 : 1 to 1.77 : 1. Oral opening antero-ventral, opening into short prepharynx. Pharynx oval; 0.05 by 0.05 to 0.05 by 0.07. Esophagus short. Gut-ceca long; posterolaterally directed; terminating a short distance from posterior extremity. Excretory pore terminal, at posterior end of body. Excretory bladder tubular reaching to level of transverse yolk-duct before bifurcating. Genital pore preacetabular, median. Genital atrium short, oval. Testis single, median, with longitudinal axis diagonally placed, overlapping ventral sucker in contracted specimens, up to 0.18 behind ventral sucker in more extended specimens, 0.16 by 0.11 to 0.20 by 0.12. Cirrus pouch large, up to 0.30 long, with armed cirrus, tubular pars-prostatica, spherical vesicula seminalis and prostatic-cells. Ovary to left of mid-line, lateral to anterior portion of testis, up to acetabular level in contracted specimens, more posteriorly in extended specimens; trilobed; 0.08 by 0.06 to 0.09 by 0.07. Ootype and Mehlis's gland median, at ovarian level. Receptaculum seminis absent. Laurer's canal probably present but not seen. Uterus emerges ventrally from ootype; proximal region acts as receptaculum seminis uterinus; descending and ascending limbs of uterus fill most of post-ovarian space. Terminal organ of uterus well developed, consisting of proximal, spherical region with sperms and a terminal spined region. Uterus enters proximal region of distal chamber. Eggs oval, 0.015 to 0.02 by 0.0125. Vitelline follicles situated in 2 lateral groups opposite posterior portion of acetabulum and a little beyond; only in highly contracted specimens are vitelline glands found opposite ovary and testis. Transverse yolk duct preovarian, giving rise to posteriorly directed yolk duct.

Host: Chaetodipterus lippei Steind.

Locality: Sekondi.

Habitat: Intestine.
Type: In the helminthological collection, Zoology Department, University College of Ghana.
No. of specimens: 15.

L. chaetodipteri may be distinguished from other species within the genus by a consideration of the following factors: In having a gut extending almost to the posterior extremity, it differs from *L. macrorchis, L. minutus, L. parvus, L. truncatus, L. himezi, L. plectorhynchi,* and *L. brevicaecum.* In having a ventral sucker appreciably smaller than the oral, it differs from *L. elongatus, L. brevicaecum, P. malasi, P. minutus* and *P. odhneri.* In having a long excretory bladder extending beyond the testis, it differs from *L. beauforti, L. longovatus, L. lintoni* and *L. mulli.* In having vitelline glands extending to acetabular level, it differs from *L. beauforti, L. longicaecum, L. plectorhynchi* and *L. cacuminatus.* In having short oval eggs, it differs from *L. bacilliovatus.* In having spines in the cirrus, it differs from *L. trifolifer.*

L. chaetodipteri resembles *L. trifolifer, L. bacilliovatus, L. longovatus,* and *L. lintoni* most closely and a re-examination of the type material of Nicoll (1915) may show that the present species is a synonym of *L. trifolifer.*

Hurleytrema trachinoti n. sp. (Fig. 7)

Diagnosis: Body roughly oval or pyriform; almost truncate posteriorly; 0.60 to 0.67 long; 0.29 to 0.30 broad at widest point in testicular region; body surface spinose. Oral sucker funnel-shaped; subterminal; large; 0.15 by 0.145 to 0.16 by 0.15. Ventral sucker smaller than oral, in posterior half of body, almost spherical, 0.10 by 0.12 to 0.10 by 0.11; ratio of oral to ventral suckers roughly 1.5:1. Oral opening subventrally situated anteriorly on oral sucker; prepharynx inconspicuous; pharynx oval, 0.08 to 0.10 by 0.07 to 0.08; esophagus short, 0.04 to 0.07 long; gut-ceca postero-laterally directed, terminating mid-way between testis and posterior extremity. Excretory pore terminal posteriorly; excretory bladder saccular, extending to testicular level before bifurcating. Genital pore slightly to left of median line, preacetabular. Genital atrium short, preacetabular. Testis single, median, compact, a short distance posterior to acetabulum, 0.09 by 0.09 to 0.06 by 0.11; vas-deferens short. Cirrus sac large, arcuate, 0.09 to 0.12 long, 0.03 broad, extending from testicular region to genital atrium, overlapping right lateral margin of ventral sucker; basal region with bipartite vesicula-seminalis; middle region with short tubular pars-prostatica followed by long, armed, eversible cirrus; remainder of cirrus pouch filled with prostatic cells. Ovary dextral, overlapping right gut-cecum, immediately behind ventral sucker, roughly oval, 0.05 by 0.06 to 0.04 by 0.06. Mehlis's gland inconspicuous; receptaculum seminis absent; descending and ascending limbs of uterus fill most of space behind ventral sucker not occupied by gonads and vitelline glands; proximal region acts as receptaculum seminis uterinus; distal region enters mid-body of terminal organ; terminal organ well developed to left of ventral sucker and overlapping it, 0.05 by 0.04 to 0.05 by 0.05, bipartite; posterior chamber spherical and unarmed; anterior chamber spinose. Eggs oval, operculate, 0.025 by 0.010 with a filament approximately twice length of egg at anopercular end. Vitelline follicles situated laterally in acetabulo-testicular region; overlapping gut-ceca; occupying an area of from 0.15 to 0.18 long; transverse yolk duct postovarian, enlarging to form vitelline reservoir from which the median yolk duct arises, beneath proximal portion of cirrus pouch.
Host: Trachinotus goreensis C. and V.
Habitat: Intestine.
Locality: Sub-littoral area of sea near Accra.
Types: In the helminthological collection, Zoology Department, University College of Ghana.
No. of specimens: Two from one host fish.

The genus *Hurleytrema* was erected by Srivastava (1938) with *H. ovocaudatum* as the type species. Two other species, namely, *H. chaetodoni* and *H. eucinostomi* were later included within the genus by Manter (1942). Later these 2 species were placed in 2 separate genera by Yamaguti (1953) in view of certain differences between them and *H. ovocaudatum* in the position of the genital pore and the vitelline glands.

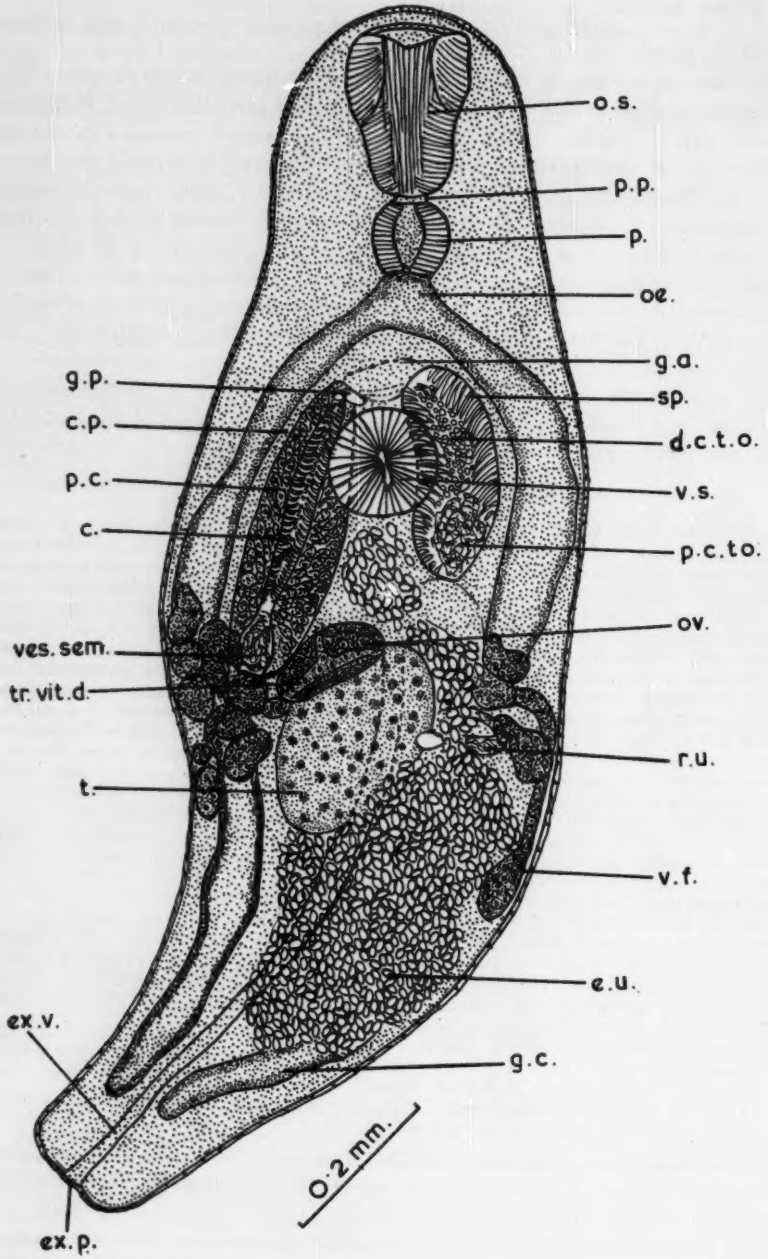

FIGURE 5. *Lasiotocus longicaecum* Manter.

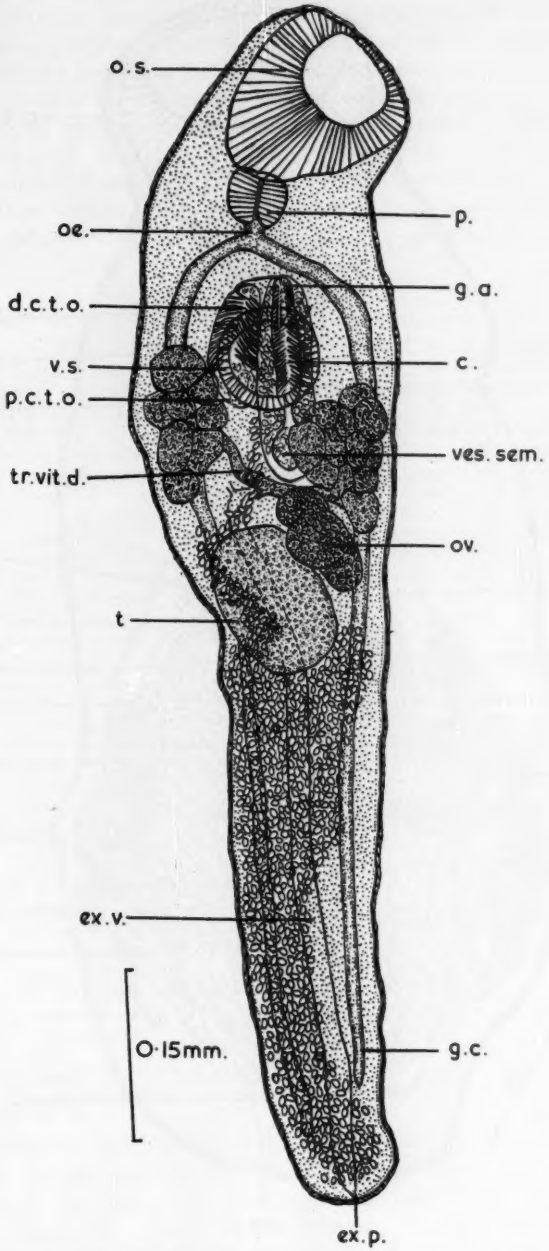

FIGURE 6. *Lasiotocus chaetodipteri* n. sp., dorsal view.

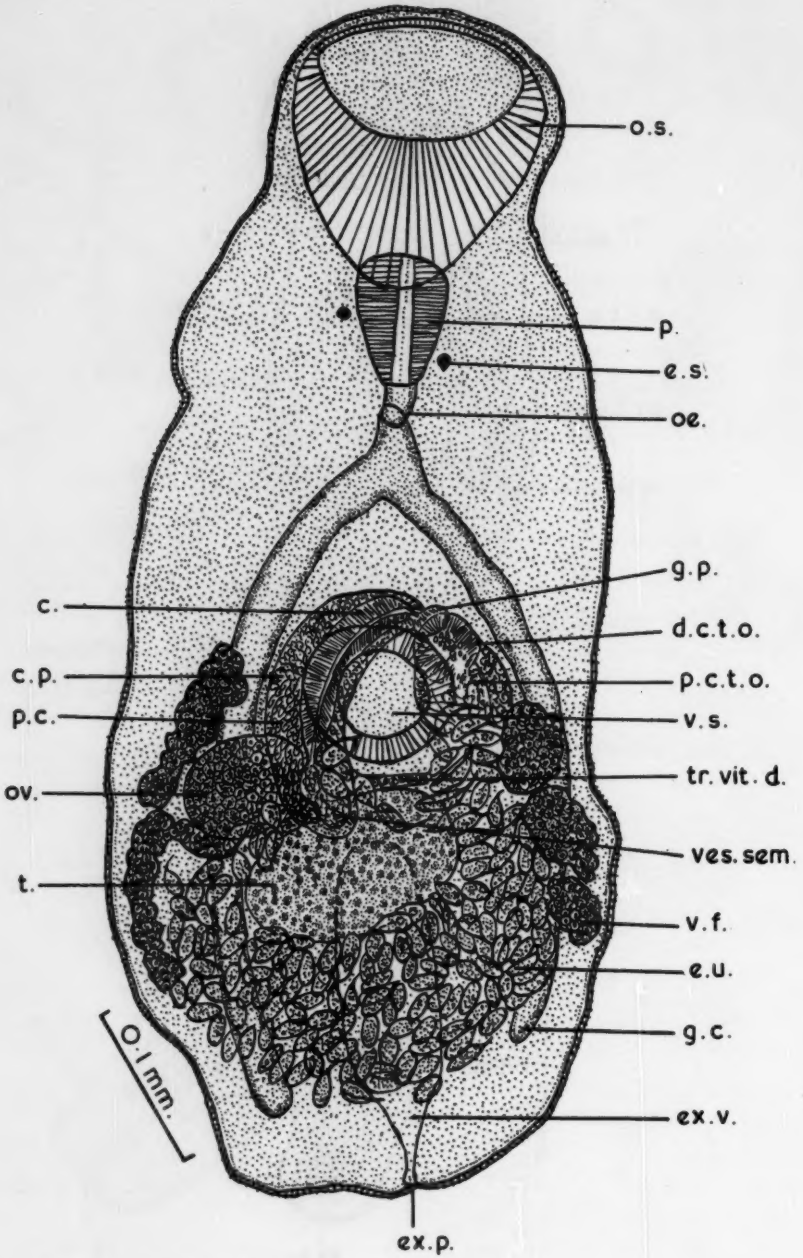

FIGURE 7. *Hurleytrema trachinoti* n. sp., ventral view.

The present species appears to be more closely allied to *H. ovocaudatum* than to the species described by Manter. *H. ovocaudatum* differs from *H. trachinoti*, however, in possessing a relatively smaller oral sucker, post-testicular medianly situated vitelline glands and a genital pore which is situated laterally at mid-acetabular level. It might be argued that the difference in the positions of the genital pores constitutes a generic difference but in actual fact the difference in position is slight. It is considered, therefore, that the erection of a new genus would be unjustified particularly in view of the apparent distortion in Srivastava's specimen as shown by the lateral position of the acetabulum. The generic diagnosis of *Hurleytrema* should, therefore, be emended to describe the genital pore as being lateral or sublateral to the acetabulum.

Diplomonorcheides magnacetabulum n. g., n. sp. (Figs. 8–10)

This species was always found in the intestine of the tongue sole, *Cynoglossus goreensis*, in association with *L. cynoglossi*. Only 1 specimen of *D. magnacetabulum* was obtained from the Accra locality compared with 5 from near Sekondi. All the specimens proved to be morphologically indistinguishable and are, therefore, considered together below.

Diagnosis: Body oval or ellipsoidal; tapering gradually at both ends; almost truncate posteriorly in contracted specimens; 0.91 to 2.34 long; 0.40 to 0.71 broad at widest point. Body surface spinulate; spines become progressively less dense posteriorly. Oral sucker sub-terminal, 0.065 by 0.08 to 0.16 by 0.20. Ventral sucker in anterior half of body, separated from oral sucker by a distance roughly equal to diameter of latter, almost spherical, 0.20 by 0.19 to 0.45 by 0.43. Ratio of oral to ventral sucker 1 : 2.3 to 1 : 2.9. Oral opening ventrally situated on oral sucker. Prepharynx short; pharynx spherical or elongate oval, 0.065 by 0.06 to 0.05 by 0.09. Gut-ceca postero-laterally directed, terminating in posterior third of distance between testis and posterior end. Excretory pore sub-terminal posteriorly. Excretory bladder tubular, dorsally situated in median line, apparently extending to level of transverse yolk duct before bifurcating. Genital pore mid-ventral, immediately in front of ventral sucker. Genital atrium thin-walled, elongated, extending to posterior level of ventral-sucker. Terminal male and female ducts lying at various levels along the atrium depending on degree of eversion of cirrus and degree of protrusion of female terminal organ. Testes paired, compact, symmetrically placed intracecally behind ovary and cirrus pouch, roughly oval, 0.15 by 0.11 to 0.27 by 0.15. Cirrus sac large, muscular, arcuate, club-shaped, containing bipartite vesicula-seminalis, a short pars-prostatica, a long armed cirrus capable of eversion and prostatic cells. Ovary to right of cirrus pouch; oval or spherical; compact; 0.105 by 0.12 to 0.17 by 0.19. Oviduct short, arising from inner-dorsal surface of ovary; ootype median. Mehlis's gland well developed. Receptaculum seminis absent; Laurer's canal present. Uterus emerging ventrally from ootype; proximal region acts as receptaculum-seminis-uterinus and is frequently dilated with sperms; ascending and descending limbs fill most of available post-ovarian space. Uterus enters the anterior chamber of a specialized bipartite terminal organ. Posterior chamber of terminal organ spherical, containing sperms; anterior chamber spinulate, with a strong muscular sphincter surrounding its opening into genital atrium. Terminal organ surrounded by deeply staining sub-cuticular cells. Eggs oval; operculate; 0.022 by 0.012 to 0.025 by 0.015. Vitelline follicles numerous, lateral in position, extending from a short distance behind ventral sucker to a short distance behind testis, overlapping gut-ceca or testis; transverse yolk duct behind ovarian level; yolk reservoir occupying median position, giving rise to median yolk duct.

Host: Cynoglossus goreensis.
Habitat: Intestine.
Locality: Sub-littoral area at Accra and Sekondi.
Types: In Zoology Department, University College of Ghana.
No. of specimens: 6.

The genera of Monorchiidae in which the testes are double include *Monorcheides* Odhner, 1905, *Paramonorcheides* Yamaguti, 1938, *Diplomonorchis* Hopkins, 1941, *Physochoerus* Poche, 1926, *Diplolasiotocus* Yamaguti, 1952, *Triganodistomum* Simer, 1929, *Palaeorchis* Szidat, 1943 and *Achoerus* Wlasenko, 1931. The present genus resembles *Paramonorcheides* most closely but differs from it in that the testes

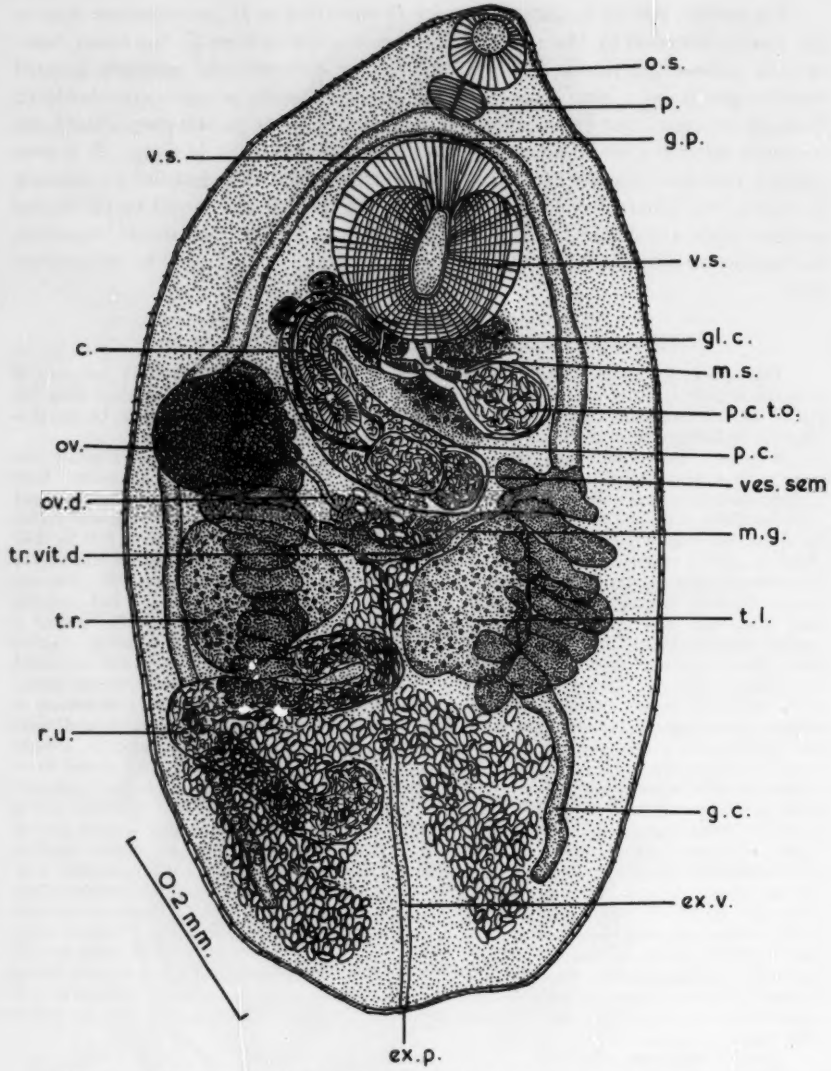

FIGURE 8. *Diplomonorcheides magnacetabulum* n. g., n. sp., ventral view.

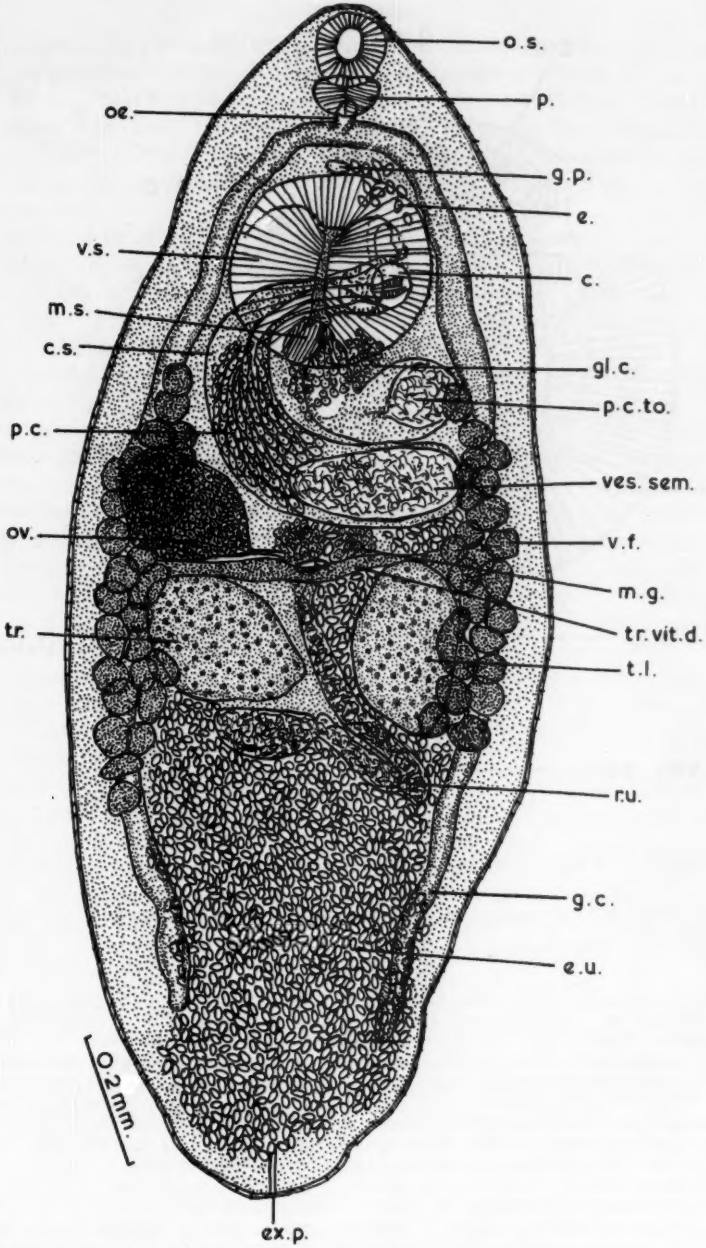

FIGURE 9. *D. magnacetabulum*, ventral view.

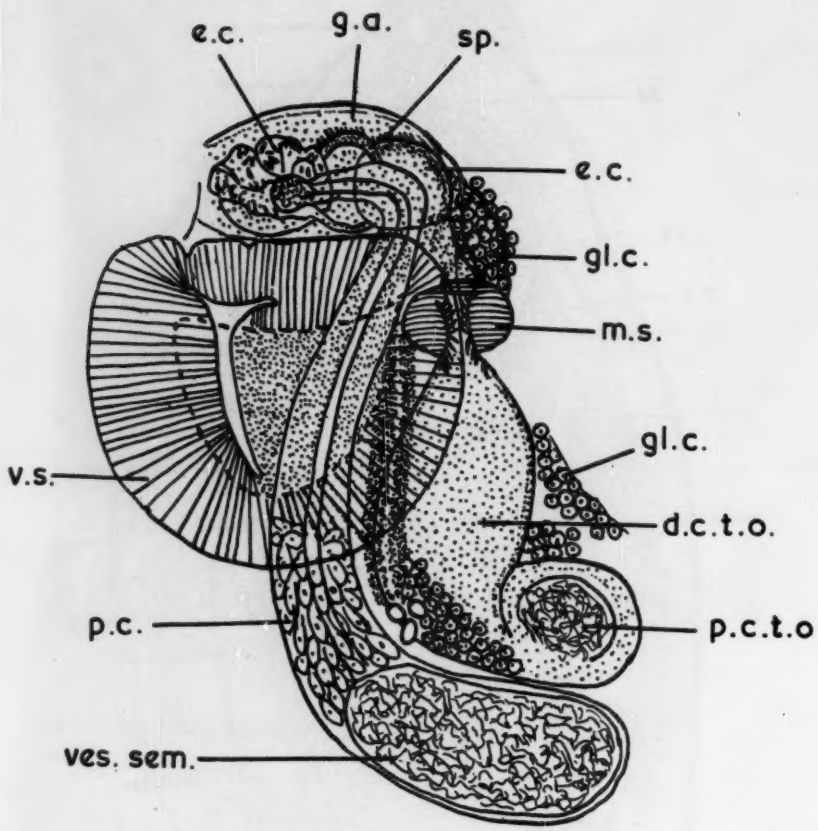

FIGURE 10. The terminal genital ducts of *D. magnacetabulum.*

are intra-cecal, the vitellaria more posterior and the genital atrium and the excretory bladder much longer.

Diplomonorcheides

Generic diagnosis: Monorchiidae Odhner, 1911. Body oval or ellipsoidal; spinulate. Oral sucker sub-terminal. Ventral suckers appreciably larger than oral sucker, pre-equatorial, close to oral sucker. Prepharynx short. Gut-ceca extending almost to posterior end of body. Excretory pore terminal posteriorly. Excretory bladder tubular, extending anteriorly to level of transverse yolk duct before bifurcating. Genital pore median; preacetabular. Genital atrium extending the length of ventral sucker. Testes symmetrical, intracecal, post-ovarian. Cirrus sac large, arcuate or S-shaped when fully extended, containing bipartite vesicula seminalis, pars prostatica and long armed, eversible cirrus. Ovary compact, to right of proximal end of cirrus. Ootype median. Receptaculum seminis absent but proximal region of uterus functions as receptaculum seminis uterinus. Laurer's canal present. Uterus extensive, entering anterior chamber of specialized bipartite terminal organ; anterior chamber spinulate with muscular sphincter at terminal end; posterior chamber spherical, usually containing sperms. Vitelline follicles numerous, in 2 lateral groups extending from behind level of ventral sucker to a point behind testis. Transverse vitelline duct postovarian.

Genotype: *Diplomonorcheides magnacetabulum.*

DISCUSSION

Five species of trematodes of the family Monorchiidae have been obtained from the intestines of fishes in the sub-littoral zone off the coast of Ghana. All the species, with the exception of *Lasiotocus longicaecum* described by Manter (1940) from *Anisotrema interruptus* (Gill) and *A. virginicus* (L.) from the Galapagos Islands and the Tortugas, Florida, are new. The present record of *L. longicaecum* from the Ghanaian marine fish *Lethrinus atlanticus,* therefore, constitutes a new geographical and host record.

The specificity of the new worms appears to be of a high order. Each one was invariably encountered in a single species of host fish although 526 individuals of 60 species of fish were examined for parasites. *L. longicaecum,* on the other hand, is less specific. Thus, Manter recorded his specimens from fishes of the family Haemulidae, whereas the present specimens were recorded from the different, though closely allied, family Lethrinidae. In general, however, the present observations support the contention of Manter (1955) that host specificity is of a high order among digenetic trematodes of marine fishes, particularly those living in warm seas.

The widespread geographical distribution of *L. longicaecum* and the remarkably close resemblance of the forms on both sides of the Atlantic with those in the Pacific indicate that this species is monotypic. Monotypy in this case is not unexpected in view of relatively low degree of host-specificity and the free-ranging habits of the host-fishes. By contrast there is some evidence that *L. cynoglossi,* a parasite of the sedentary tongue-sole, may be a polytypic species. The differences between the populations of this species from the Accra and Takoradi areas is probably genotypic rather than phenotypic in nature as the host fish is the same in both cases. Further, it would appear that these differences are not caused by a variation started by a single fertilized egg but are of a more generalized nature as the parasites were found in several host fishes. It is hoped that further sampling will become possible to verify the extent of this trend.

In the case of *Diplomonorcheides magnacetabulum* it has already been shown that the form from the vicinity of Accra is indistinguishable from those in the Sekondi area although occurring in the same host fish as *L. cynoglossi.* This apparent anomaly may be due to the fact that *D. magnacetabulum* is less subject to change than *L. cynoglossi* or alternately that it is of more recent origin.

The origin of species or the formation of polytypic species amongst digenetic trematodes would be favored by the isolating effect resulting from the relative immobility of either the definitive or the molluscan intermediate host. In the present instance the immobility of the definitive host may well be important. The high order of specificity shown by digenetic trematodes and their peculiarity in requiring 2 hosts including the relatively inert mollusc for the completion of the life-cycle would appear to enhance the prospects of isolation and hence speciation, despite the apparently constant environment in which the parasites live.

SUMMARY

1. Five species of digenetic trematodes including 1 previously described species, 1 new genus and 3 new species are describd from Ghanaian sub-littoral marine fishes. They are as follows: *Lasiotocus longicaecum* Manter 1940, from *Lethrinus atlanticus* C. & V.; *Lasiotocus cynoglossi* n. sp. from *Cynoglossus goreensis* Steind.;

Lasiotocus chaetodipteri n. sp. from *Chaetodipterus lippei* Steind.; *Hurleytrema trachinoti* n. sp. from *Trachinotus goreensis* C. & V.; and *Diplomonorcheides magnacetabulum* n. g., n. sp. from *Cynoglossus goreensis* Steind. The populations of *L. cynoglossi* n. sp. from the Accra and Sekondi areas show differences which are considered to be sub-specific. The sub-species from the Accra area is named *L. cynoglossi magniovatus* n. sp., n. sub. sp. and that from the Sekondi area *L. cynoglossi major* n. sp., n. sub. sp.

2. The present record of *L. longicaecum* provides new geographical and host records.

3. The possible synonymy of the allied genera *Lasiotocus* Looss, 1907, *Proctotrema* Odhner, 1911, *Genolopa* Linton, 1910, *Paraproctotrema* Yamaguti, 1934, and *Proctotrematoides* Yamaguti, 1938, is discussed. It is concluded that the genera *Genolopa* and *Proctotrematoides* should be retained unless it can be proved that the spines described as being atrial spines in the type-species are in reality the spines of the genital ducts. With the exception of the type species of *Genolopa* and *Proctotrematoides* and *G. fusiforme* (Yamaguti, 1934) and *G. lethrini* (Yamaguti, 1953) all other species allocated to the genera listed above should be included in the genus *Lasiotocus*.

4. With the exception of *L. longicaecum* the trematodes found during the present investigation appeared to show a high order of host-specificity.

5. It is suggested that *L. longicaecum* may be a monotypic species and *L. cynoglossi* a polytypic one. The origin of polytypy in the latter species would be favored by the isolating effect resulting from the relative immobility of the definitive host.

6. It is suggested that the high specificity of the digenetic trematodes and their peculiarity in requiring 2 hosts including the relatively inert mollusc for the completion of their life cycle would appear to enhance the prospect of isolation and hence speciation despite the apparently constant environment in which the parasitic stages live.

ACKNOWLEDGMENTS

The author is indebted to the Fisheries Department, Accra and the Government Prison Fisheries for assistance in obtaining the fish hosts and to the University College of Ghana for financial aid.

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Key to lettering of figures.

c.—cirrus; c.ch.—central chamber of Mehlis's gland; c.m.—circular muscles; c.p.—cirrus pouch; c.s. cirrus sac; d.c. t.o.—distal chamber of terminal organ; e.c.—everted cirrus; e.s.—eye spot; e.u.—eggs in uterus; ex.p.—excretory pore; ex.v.—excretory vesicle; g.a.—genital atrium; g.c.—gut cecum; g.p.—genital pore; gl.c.—gland cells; l.c.—Laurer's canal; m.g.—Mehlis's gland; m.s.—muscular sphincter of terminal organ of uterus; o.s.—oral sucker; oe.—esophagus; ov.—ovary; ov.d.—oviduct; p.—pharynx; p.c.—prostatic cells; p.c.t.o.—proximal chamber of terminal organ; p.p.—prepharynx; r.u.—receptaculum uterinus; sp.—spine; t.—testis; t.l.—left testis; t.o.—terminal organ of uterus; t.r.—right testis; tr.vit.d.—transverse vitelline duct; v.f.—vitelline follicles; v.s.—ventral sucker; ves.sem.—vesicula seminalis; vit.res.—vitelline reservoir.

AMERICAN SOCIETY OF PARASITOLOGISTS

PRELIMINARY ANNOUNCEMENT OF THE THIRTY-FOURTH ANNUAL MEETING

Sunday, Monday, Tuesday, and Wednesday, August 30–September 2, 1959
Pennsylvania State University, University Park, Pennsylvania

In accordance with the action of the Council, the American Society of Parasitologists will convene for a 4-day program in conjunction with the meeting of the American Institute of Biological Sciences.

The program will be arranged in the same manner as in previous years. The Council will meet at 2 P.M. on Sunday, August 30. Contributed papers to be read will be scheduled on Monday and Tuesday, August 31 and September 1. The presidential address, annual luncheon, annual business meeting, and demonstration session will be scheduled on Wednesday, September 2.

The call for papers is being sent to members of the Society on February 5. In order that the program outline be available for the AIBS Bulletin and that the abstracts be printed and mailed to the membership before the meetings, it is necessary that requests for program space be received in the office of the Secretary not later than April 15, 1959. Members are hereby reminded of this date, and are requested to be prompt in the submission of papers for the program.

Information concerning registration, housing arrangements, food services, and an outline of the program will appear in the AIBS Bulletin.

PAUL. E. THOMPSON
Secretary

BIVESICULA TARPONIS, A NEW TREMATODE IN THE TARPON, *MEGALOPS ATLANTICUS* (Cuv. & Val.), FROM THE WEST COAST OF FLORIDA.

FRANKLIN SOGANDARES-BERNAL AND ROBERT F. HUTTON

Florida State Board of Conservation Marine Laboratory,* Maritime Base, Bayboro Harbor, St. Petersburg, Florida

This study was made in connection with a program on parasites and diseases of marine animals which is currently being sponsored by the Florida State Board of Conservation.

In view of the importance of the tarpon as an important local and tourist attraction along the west coast of Florida and especially in the Tampa Bay area, a special attempt was made to secure specimens for examination. Three recently dead tarpons were obtained and a new trematode, described below, was found in the pyloric ceca and along the entire length of intestine of all 3 specimens.

Unless otherwise cited, all measurements are in millimeters.

Bivesicula tarponis n.sp.
(Figs. 1 to 4)

Host: Megalops atlanticus (Cuv. & Val.); tarpon; family Megalopidae
Incidence of infection: In 3 of 3
Location: Pyloric ceca and entire length of intestine.
Locality: Gulf of Mexico 1 mile off Gulf Beaches, City of St. Petersburg Beach, Florida
Holotype: U. S. Nat. Mus. Helm. Coll. No. 38386.

Diagnosis (based on 9 specimens; measurements on 4 favorable specimens): *Bivesicula;* body elongately elliptical, convex dorsally, 1.690 to 2.444 long by 0.884 to 1.144 wide. Cuticle unspined; body spines may have been lost during fixation. Fine pigment flecks present between oral sucker and cecal junction. Mouth terminal. Oral sucker 0.055 to 0.082 long by 0.064 to 0.073 wide. Pharynx absent. Esophagus about 7 times longer than oral sucker. Ceca 2, narrow, extending to about 4/5 length of body, posterior to testis. Genital pore median, equatorial. Testis single, median, intercecal, immediately post-equatorial, roundish in shape; 0.319 to 0.573 long by 0.364 to 0.455 wide. Two *vasa efferentia* beginning at testis and extending anteriorly between ceca and cirrus sac, one on each side of body, to enter external seminal vesicle at anterior border of cirrus sac. Cirrus sac extending from genital pore towards anterior end of body, confined to intercecal area between testis and cecal junction, 0.346 to 0.510 long by 0.273 to 0.337 wide; with external seminal vesicle at anterior tip; internal seminal vesicle occupying anterior 1/3 sac, connecting with muscular cirrus at its middle (Figs. 1 and 2) or anterior end (Figs. 3 to 4), depending upon contraction of cirrus. Prostate cells surrounding all structures within cirrus sac. Ovary roundish, dextral to midline, level with anterior edge of testis and posterior tip of cirrus sac; 0.155 to 0.182 long by 0.137 to 0.182 wide. Vitellaria extending halfway from oral sucker to cecal junction, coalescing in area anterior to cirrus sac, overlapping ceca along posterior course to end from middle of testis (in one extremely distended specimen), to a short distance posterior to testis. Vitelline receptacle large, on sinistral side of ovary, ventral between testis and ovary. Uterus descending between testis and ceca on dextral side of body, behind testis to near posterior end of body between excretory stems; ascending on same side of descent, to enter genital atrium at its median posterior border. One loop of uterus behind testis on dextral side of body forms a *receptaculum seminis uterinum.* Eggs thick shelled; uncollapsed from 57 to 74 microns long by 42 to 50 microns wide; collapsed eggs from 55 to 86 microns long by 31 to 40 microns wide. Excretory vesicle with 2 dorsal, intercecal, wide stems, one on

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* Contribution No. 14.

each side of body; extending from a short distance anterior to excretory pore to end about ½ distance from cecal junction and oral sucker.

DISCUSSION

There are 5 species in the genus *Bivesicula* Yamaguti, 1934. These species are: *B. australis* Crowcroft, 1947; *B. claviformis* Yamaguti, 1934, genotype; *B. epinepheli* Yamaguti, 1938; *B. hepsetiae* Manter, 1947; and *B. synodi* Yamaguti, 1938.

B. hepsetiae is known from Tortugas, Florida and Puerto Rico, while *B. claviformis, B. epinepheli,* and *B. synodi,* are from Japan. *B. australis* is known only from Tasmania.

B. tarponis differs from *B. claviformis, B. epinepheli* and *B. synodi* by lacking a pharynx and possessing a very long esophagus as compared with pharynx present and esophagus shorter than oral sucker.

B. tarponis differs from *B. claviformis, B. epinepheli, B. hepsetiae,* and *B. australis* by possessing vitellaria which extend posterior to testis as compared with vitellaria to anterior border of testis. It further differs from *B. claviformis, B. epinepheli, B. hepsetiae* and *B. synodi* by possessing ceca which end posterior to testis as compared with ceca ending at anterior border of testis. In addition, *B. tarponis* differs from *B. australis* by possessing an equatorial genital pore and relatively smaller oral sucker (1/6 width of testis) as compared with postequatorial genital pore and larger oral sucker (about ¾ width of testis). *B. tarponis* also differs from *B. synodi* by possessing vitellaria which extend anteriorly only to mid-esophagus as compared with vitellaria to oral sucker.

Le Zotte (1952, 1954) and Cable (1956) have reported what they believe to be bivesiculid larvae from Puerto Rico. Hutton (1952) reported a similar larva from *Cerithium eburneum* in the Miami area. Recently one of us, (R.F.H.), collected a cercaria from a plankton haul in Bayboro Harbor, St. Petersburg, Florida, a site where many tarpon have been observed rolling. The cercaria (Fig. 5) is apparently identical with that reported by Hutton (1952). The presence of tarpon and the bivesiculid cercaria in one location does not preclude that the cercaria may be the larva of *B. hepsetiae* or of some other bivesiculid. According to Briggs (1958) *Atherinomorus stipes* (Müller and Troschel) (= *Hepsetia stipes* (Muller & Troschel)), the only known host of *Bivesicula hepsetiae,* does not occur so far north as St. Petersburg, Florida. Dr. Victor G. Springer, Ichthyologist, of this laboratory has never collected *A. stipes* in the vicinity of St. Petersburg. This larva may be that of *Bivesicula tarponis. Cerithium algicola* exists in Bayboro Harbor, but in limited numbers.

Manter (1954) mentioned that the genus *Bivesiculoides* Yamaguti, 1938 may be a synonym of *Bivesicula* but did not synonymize the genera. The only apparent difference between the two genera seems to be that the uterus is completely pretesticular in *Bivesiculoides.* We concur with Manter's (1957) views and believe it best to retain *Bivesiculoides* Yamaguti, 1938 until intermediate material justifies a synonymy of the two genera.

Both Cable (1956) and Le Zotte (1954) have related the bivesiculids to the Azygiidae, a view with which we concur for the present time.

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———1954 Studies on marine digenetic trematodes of Puerto Rico: The family Bivesiculidae, its biology and affinities. J. Parasitol. **40**: 148–162.

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EXPLANATION OF FIGURES

FIGURE 1 was drawn with the aid of a camera lucida. The projected scale represents millimeters. Figures 2 to 4 are sketches. Figure 5 is a photomicrograph. Abbreviations used: C. cirrus; ESV, external seminal vesicle; GA, genital atrium; GP, genital pore; ISV, internal seminal vesicle; UT, uterus; VE, vas efferentia.

PLATE I

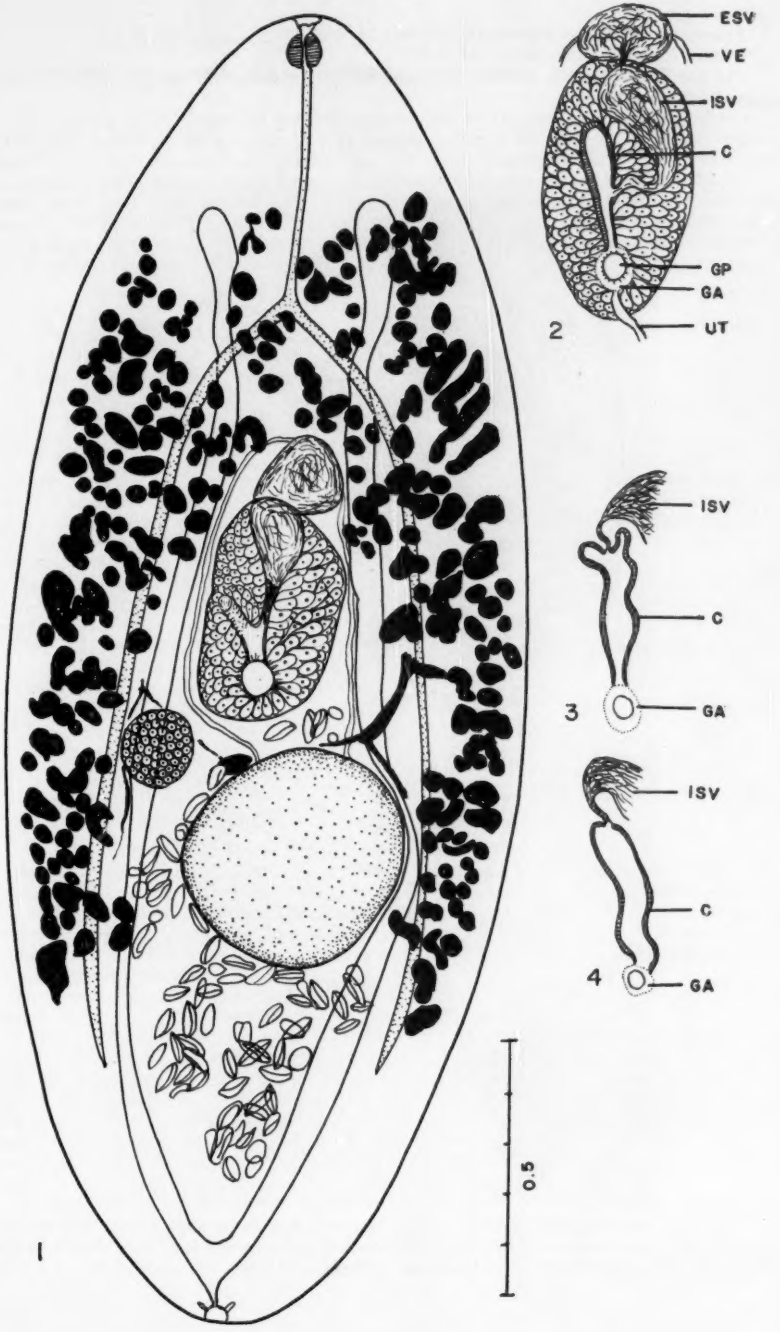

FIGURE 1. *Bivesicula tarponis*, ventral view of holotype.
FIGURE 2. Same, cirrus sac of paratype.
FIGURES 3 to 4. Same, showing cirrus in different stages of contraction and insertion of internal seminal vesicle.

PLATE II

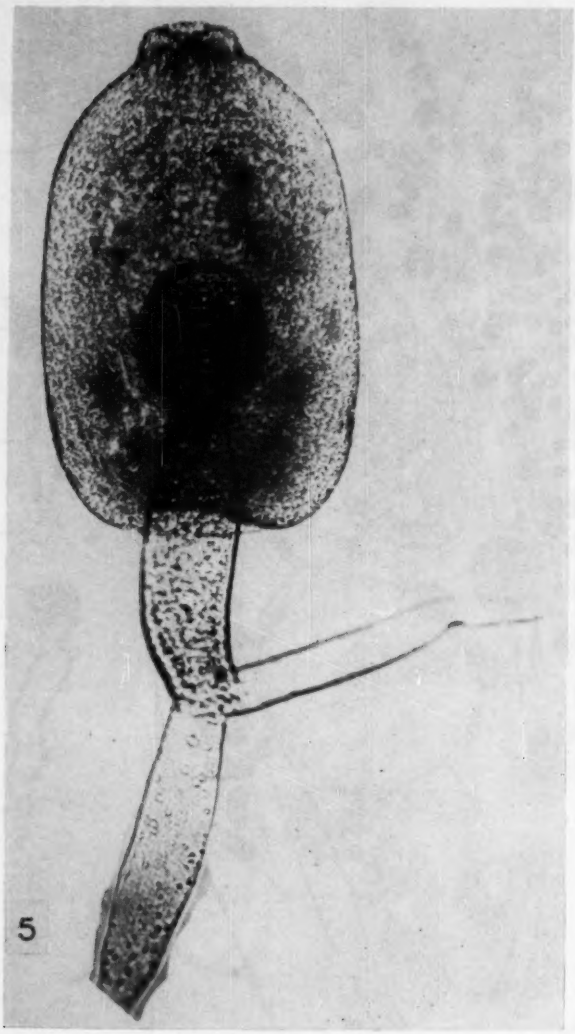

FIGURE 5. Photomicrograph of bivesiculid cercaria reported by Hutton (1952) from Miami, Florida, and which is apparently identical with the one taken by the same individual in a plankton haul from Bayboro Harbor, St. Petersburg, Florida.

AMERICAN SOCIETY OF PARASITOLOGISTS

Forty-Eighth Council Meeting, Bloomington, Indiana, August 24, 1958

The meeting of the Council of the American Society of Parasitologists was called to order by President Arthur C. Walton at 2:15 P.M., Sunday, August 28, 1958, in the Union Building, Indiana University, Bloomington, Indiana. In addition to President Walton, the meeting was attended by President-Elect A. O. Foster, Vice-President R. M. Cable, Past Presidents J. E. Ackert, E. R. Becker, G. F. Otto, and E. W. Price, Treasurer R. M. Stabler, the Secretary, Councilors W. S. Bailey (ex-officio), G. H. Ball, P. C. Beaver, L. Jacobs (ex-officio), F. J. Kruidenier, D. A. Porter, and E. D. Wagner (non-voting), and Committee Representatives J. M. Andrews, M. Doss, and A. McIntosh and N. D. Levine.

I. Reports of Officers and Members of the Council

1. *President* (*A. C. Walton*) : No report.
2. *President-Elect* (*A. O. Foster*) : No report.
3. *Vice-President* (*R. M. Cable*) : No report.
4. *Secretary* (*P. E. Thompson*) : As of August 4, 1958, the Secretary's records show that there are 1040 names on the rolls of the Society of whom 934 are active members in good standing, 8 are non-subscribing Inactive Members, 43 are delinquent as of this date for 1958 dues, 35 are delinquent for both 1957 and 1958 dues, 8 are Emeritus Members, and 13 are Honorary Members. Since the last meeting, notices of the deaths of 3 members have been received, 7 members have resigned, and 24 were dropped as of January 1, 1958 for non-payment of dues. In the same period of 9 months, 63 active members, 5 Emeritus Members, and 3 Honorary Members have been elected to the Society. Later in this meeting, the names of 10 applicants will be submitted as candidates for election to active membership.

Special problems handled by this office during the year included: (1) Through the AIBS and the Office of Naval Research, arranged for roundtrip transportation for 5 members of the Society to the Sixth International Congresses of Tropical Medicine and Malaria in Lisbon; (2) Through the devoted efforts of Mrs. Gerry Jacob, all Society's Archives through 1957 have been indexed and bound.

The report was accepted and ordered placed on file.

5. *Treasurer* (*R. M. Stabler*) : The report submitted for the 9-month period November 1, 1957 to August 1, 1958 is summarized as follows:

Balance on Hand, November 1, 1957	$ 3,479.14
Collections to August 1, 1958	15,351.98
Total Funds for 1958	$18,831.12
Total Expenditures for 1958	14,395.62
Total Cash Balance August 1, 1958	$ 4,435.50

In submitting his report, the Treasurer pointed out that during his fourteen year tenure the membership and number of copies of the Journal have increased two-fold but that costs have trebled. He also pointed out that the working balance has continuously declined during the past 3 years, but that the increase in dues and subscription rate, effective as of 1959, should start the climb back to greater solvency. (Bills amounting to approximately $3,000 are yet to be paid out of the August 1, 1958 balance.)

The report, having been audited by Certified Public Accountants, Trichak and Trainor, Colorado Springs, Colorado, was accepted and ordered placed on file.

6. *Editor* (*L. Jacobs*) : The costs per page of the Journal, including total printer's costs, wrapping, and mailing, for the years 1956, 1957, and 1958 (first 3 issues) were $17.69, $17.45, and $19.17, respectively. During the period November 1957–June 1958, 126 papers were processed; of these 72 were acceptable with little or no modification, 50 were returned for revision, and 4 were either rejected or withdrawn. The Editor will continue to serve until Volume 44 is completed, after which Dr. E. R. Becker will assume full responsibility. The Editor expressed appreciation for the cooperation of the Associate and Assistant Editors and the Treasurer of the Society.

Dr. Jacobs expects to continue to be active in the AIBS-sponsored Conference of Biological Editors. The latter group is seeking funds from the National Science Foundation for a careful study of the business management of certain societies that have indicated an interest in joint management of journals and other business matters. The American Society of Parasitologists

expressed an interest in this 2 years ago. If the study shows that it is feasible to organize a central office capable of handling such matters for the interested Societies, a grant will be requested to provide funds to establish and equip such an office and provide funds for its first year of operation. The Society will be kept aware of all developments.

The report was accepted and ordered placed on file.

7. *Custodian (W. S. Bailey)*: The report submitted for the ten-month period October 1, 1957 to August 1, 1958 is summarized as follows:

Balance on Hand, October 1, 1957	$4,170.58
Net Collections During Period	2,687.77
Expenditures During Period	1,209.77
Balance on Hand, August 1, 1958	$5,648.58

The report was accepted subject to the report of the Auditing Committee and ordered placed on file.

8. *Report on Endowment Fund (N. R. Stoll)*: This report covers the period October 31, 1957 to August 15, 1958.

	1957	Interest Earned	1958
Savings Account*	$ 489.62	$16.03	$ 505.65
U.S. Savings Bond*	861.00	26.00	887.00
	$1350.62	$42.03	$1392.65

* No. 5197, Princeton Savings and Loan Assn.

* No. M1 681 662F of $1000.00, issued January, 1950 at purchase price of $740.00

The report was accepted subject to certification of the Fund by selected members of the Society that reside in the New York area.

II. Reports of Standing Committees

1. *Committee on Visual Education and Instruction (M. S. Ferguson, Chr., D. V. Moore, J. C. Swartzwelder)*: It was expected that the revision and publication of the catalog of parasitological films would have been completed several months ago. Because of the time required to contact all sources of films to verify their availability and to determine if new titles can be added, the list is not quite completed. It is now being assembled and will be ready for distribution to the membership by fall.

During the year the Committee has answered many requests for information on parasitological films. The volume of requests indicates a continuing interest in these visual aids.

The report was accepted and ordered placed on file.

2. *Committee on Nomenclature and Terminology (A. McIntosh, Chr., G. W. Hunter III, F. J. Kruidenier, N. R. Stoll, G. W. Wharton)*: The functioning and report of the Committee dealt with the results obtained by participation of 3 of the members (A. McIntosh, N. R. Stoll, G. W. Wharton) and several other members of the Society in the Colloquium on Zoological Nomenclature and the Zoological Nomenclature Section of the XVth International Congress of Zoology, London, England July 9–23, 1958. These representatives of the Society were instrumental in having inserted into the new English draft of Règles Internationales de la Nomenclature Zoologique (as revised by the 1948 Paris and 1953 Copenhagen Congresses) the substance of the following American Society of Parasitologists Resolution on Collective Groups (adopted during the 1956 annual meeting of the Society).

"Certain biological groups which have been proposed distinctly as collective groups, not as systematic units, may be treated for convenience as if they were genera, but they require no type species. Any specific name proposed in combination with a collective group name shall have the same status as if it had been proposed in combination with a generic name of a systematic unit". (This resolution would be in keeping with the practice of helminthologists for more than 50 years.)

No mention of this resolution had been made in the first published draft of the Code, but an opportunity to have the Collective Groups Resolution inserted arose through the redrafting of the Code. Dr. Chester Bradley (Cornell University) proposed inclusion of the substance of the Resolution and the Society representatives, along with others, succeeded in having the proposal sanctioned. The above decision of the Colloquium was subsequently adopted by the International Commission of Zoological Nomenclature.

The report was accepted and ordered placed on file; and the Council expressed its appreciation to the Committee for success in defending the Collective Groups Resolution of the Society.

3. *In Memoriam Committee (M. A. Doss, Chr., E. Chernin, M. Voge)*: The Committee noted the names of 3 members of the Society who have passed away since the 1957 meeting of the Council.

These names appear on page 48 of the Annual Supplement and Program of 1958.

The report was accepted with a deep sense of loss to the Society and ordered placed on file.

4. *Public Relations Committee* (*H. W. Brown, Chr., A. C. Cuckler, B. H. Kean*): No report.

III. Reports of Representatives and Delegates

1. *To Council of the A.A.A.S.* (*J. M. Andrews, G. W. Wharton*): As there were no ASP issues to be brought before the Council of the A.A.A.S., the function of the Representative has been to select and transmit information concerning the A.A.A.S. which may be of interest or concern to the Council of ASP.

As of January, 1958, the merger of "Science" and the "Scientific Monthly" was activated. Simultaneously with this event, the annual dues of the A.A.A.S. went from $6.50 to $8.50.

An important event of the year was the meeting of the *Parliament of Science,* suggested to the A.A.A.S. Board of Directors by the A.A.A.S. Council to discuss topics of current importance to science in the U.S.A. This was held on March 15–17 in Washington under the Chairmanship of Warren Weaver, and considered such topics as the support of science, the organization and administration of the Nation's scientific effort, communication among scientists, the selection, guidance, and assistance of students, and the improvement of teaching and education. It was supported by $10,000 grants from the Rockefeller Foundation, and from the Sloan Foundation.

As of December 31, 1957, A.A.A.S. membership stood at 55,727; a gain of 3,009 for the year. Its income for that year was $832,000 which exceeded its expenses for the same year by $8,600.

Future meeting places are planned as follows: December, 1959—Chicago (instead of Denver, as the Zeckendorf Hotel in Denver will not be completed as planned in time for the 1959 meeting); December, 1960—Philadelphia (no change); December, 1961—Denver (instead of Milwaukee); December, 1962—Boston (no change); December, 1963—Milwaukee.

The ASP Representative to the A.A.A.S. Council will be glad to transmit any message or proposal to the A.A.A.S. Council for or in behalf of the ASP at the forthcoming A.A.A.S. Council meeting in Washington in December, 1958.

The report was accepted and ordered placed on file.

2. *To Governing Board of A.I.B.S.* (*A. C. Walton; L. J. Olivier,* alternate): The semi-annual meeting of the Governing Board of the American Institute of Biological Sciences was held May 9 and 10, 1958, at the University of Wisconsin, Madison, Wisconsin, with a majority of the member Societies represented.

Dr. Cox presented a progress report on the activities of the A.I.B.S. since the Stanford meeting of last August. Among items of interest to our Society was the fact that of the over $400,000.00 budget for the year, only $33,000.00 was unrestricted for use by the A.I.B.S. in its various services. Of this amount only $17,000.00 came from dues and the remainder came from balances after various contractual services had been completed. A deficit of $4,000.00 has accumulated and means for removing this deficit involves a recommendation that the by-laws be changed to require that each Society pay $1.00 per capita on its membership. There are several Societies with memberships well above the present 1,000 limit ($1,000.00 is now the dues limit per society). Our Society is requested to vote on such a change. Inasmuch as this change would not affect our Society for several years to come, your representative recommends approval. Some of the larger Societies have already agreed to such a change, but a vote from all groups involved is necessary before such a change can be initiated. A second point was the approval of application for membership of several Societies, among them the very large group of Entomologists. If the amendment passes, the new dues income would be increased by $7,000.00 and not only remove the deficit but also give a small working balance for the future. Dues for the affiliated Societies should also be based on membership rather than on a flat fee. It is recommended that we endorse this change in the by-laws as well as the change with regard to dues of the regular members. No plan of such fee changes has as yet been presented, pending approval by member groups. Affiliates do not vote, and the general consensus was that they should not be given such a vote as long as such affiliate groups outnumber full-membership Societies. Plans to change the Bulletin to 6 numbers per year, progress of the Quarterly Journal of Biology, increased translations of Russian Journals, sponsorship of College Speakers' Bureau and of a possible H.S. Speakers' Bureau, progress on Joint Editorial activities for varoius Biological Journals, and effects of the A.I.B.S. protest to Civil Service concerning pay of Biologists were discussed. A full report on these activities will be published in the A.I.B.S. Bulletin.

Another activity of the A.I.B.S. was the help afforded in getting travel assistance to various International Congresses. Our Society benefits to the extent of O.N.R. assistance for 5 of our members to attend the International Congress at Lisbon through transportation by MATS. Another item of special interest to us is the granting of $3410.00 to the Society toward the expenses of the new Journal of Parasitology Index. A.I.B.S. cooperated to a valuable degree in getting this grant.

Many other items of interest were discussed at the 2-day session and referred for further study and report at the Bloomington Meeting. One such item was the request that our Council consider approval of a change in the A.I.B.S. Constitution adding 4 members-at-large to the Governing Board. These men would serve 4-year terms, with rotational system being set up by shortening the first appointment appropriately so that one new member would be appointed each year after the first. Such members-at-large would not necessarily come from Societies now in A.I.B.S., but might be outstanding Biologists from any field of activity.

The report was accepted and ordered placed on file.

3. *To Division of Biology and Agriculture of the National Research Council* (*N. D. Levine; W. S. Bailey,* Alternate): The annual meeting of the Division and the first full meeting of the NRC in its 40-year history were held in Washington, D. C., on March 30–April 1, 1958. The activities of the Division are being curtailed because of shortage of funds. The Biology Council has been discontinued and the Committee on Educational Policies has been inactivated.

The report of the *ad hoc* Committee on Organization of College Courses in Parasitism was published in the February 1958 issue of the *Journal of Parasitology.*

The Food and Nutrition Board plans to issue 4 technical reports in 1958. The Food Protection Committee has organized a Subcommittee on Carcinogenesis. The Pesticides Committee has prepared a list of chemicals used in insecticides, fungicides, herbicides, nematocides, plant growth regulants and molluscicides in production of food.

Several committees of the Agricultural Board are working in areas of interest to parasitologists. Included among these are an evaluation of methods for determining losses caused by insects, nematodes, plant diseases, vertebrate pests, and weeds. Other areas are systemic insecticides and the biological control of soil-borne plant pathogens.

The Institute of Laboratory Animal Resources has prepared a set of standards for mouse-production. It is preparing similar standards for experimental rats, also guides for the shipment of research dogs and for the care and maintenance of animals. A revised Handbook of Laboratory Animals is being prepared. "Information on Laboratory Animals" began to appear as a quarterly newsletter in 1957. Subcommittees will be established on Cell Culture and on Germ-Free and Pathogen-Free Animals.

The program of Handbook publications has been revised; present plans call for publication of the Handbook of Respiration in June, 1958, Handbook of Fungicides in October, 1958, Handbook of Insecticides in December, 1958, and the Handbook of Circulation in June, 1959.

The stimulation of interest in biology and the improvement of biology teaching were discussed. Summer institutes and refresher courses at scientific meetings were mentioned. The A.I.B.S. hopes to procure funds for a study directed toward improvement of the teaching of biology in high schools.

A request by the University of Michigan for NRC support of "A Center for Tropical Studies on the Isthmus of Tehuantepec, Mexico" was not endorsed by the Division because the proposed Center would be administered solely by the University.

The report was accepted and ordered placed on file.

4. *To the Agricultural Research Institute* (*C. M. Herman*): No report could be given because the A.R.I. had not met since our Philadelphia meeting.

5. *To National Council on Trichinosis* (*W. H. Wright*): No report as there had been no meeting of that Council since our Philadelphia meeting.

6. *Of Southern California Parasitologists* (*E. D. Wagner*): Five regular meetings were held during the past academic year. In addition, our members were invited to attend a special series of talks by Professor Cecil A. Hoare and Dr. R. Elsdon-Dew which were sponsored by the Departments of Zoology, Infectious Diseases and Entomology of the University of California, Los Angeles. A copy of the announcements for each of these meetings is appended to this report.

The year was marked by adoption by the Council of the American Society of Parasitologists of our organization as its first affiliate. This action was taken at its Forty-Seventh Council Meeting, October 30, 1957 in Philadelphia. Dr. Gordon Ball, our elected representative at the Council Meeting amplified on the application and served, appropriately, as spokesman for the Southern California Parasitologists. Dr. Ball was elected to the ASP Council at the same meeting, so Dr. Edward D. Wagner was selected to serve as the non-voting SCP representative for the Forty-Eighth Council Meeting at Bloomington, Indiana in August, 1958.

After considerable effort and negotiation by Drs. Kessel and Ball, and after the SCP so requested at its March Meeting, formal invitations were extended to The American Society of Parasitologists and The American Society of Tropical Medicine and Hygiene to hold their joint meeting in Los Angeles in 1960.

The report was accepted and ordered placed on file.

IV. Reports on Special Committees

1. *Committee on a Career Booklet* (*D. V. Moore, Chr., R. M. Cable, A. C. Chandler, D. L. DeGuisti, C. G. Goodchild, G. L. Hoffman, D. W. Micks, D. A. Porter, R. E. Thorson*): No report.

2. *Committee on a Research Award* (*P. C. Beaver, Chr., L. A. Stauber, D. B. McMullen*): As directed by council action at its 1957 meeting in Philadelphia, a committee was appointed by the President, to consider the desirability and feasibility of establishing an award in recognition of meritorious contributions in the field of parasitology. The Committee reported that it deemed an award desirable, and that sponsorship of such an award is available to the society as follows: Parke, Davis and Company has offered to sponsor a research award in parasitology, to be administered by the American Society of Parasitologists, made annually or less often as the Society sees fit, and consisting of a medallion, a check for a thousand dollars, and one hundred and fifty dollars for travel allowance for the recipient. In recommending that the award be sponsored, Parke, Davis and Company had in mind a medal similar to that which accompanies the Bailey K. Ashford Award in Tropical Medicine with respect to identifying the sponsor, the medal to be designed and named by the Society. The hope was expressed that the award would be used in recognition of work deemed meritorious by the Society in any phase of parasitology.

The Committee considered the offer of Parke, Davis and Company to be entirely adequate, and recommended that the offer be accepted along with an expression of appreciation of the generosity of Parke, Davis and Company in providing sponsorship of an award. The Committee also made the following recommendations. The award shall be named in honor of Henry Baldwin Ward. It shall be given annually, or less often, to an outstanding member of the Society for meritorious research or services to the Society, accomplished within 15 years after completion of academic training. The recipient shall be selected by a "Committee of Awards." The Committee shall consist of 5 members who represent the several disciplines within the Society. They shall be appointed by the President for 2-year terms of office, except for 2 of the first appointees, who shall serve 1 year so as to permit continuity within the Committee. No members of the Committee shall be eligible for consecutive appointment. The Chairman of the Committee for each year shall be designated by the President.

The report was accepted and ordered placed on file.

3. *Committee to Nominate a Candidate for Editor* (*P. D. Harwood, Chr., G. H. Ball, A. C. Chandler, L. E. Rozeboom*): The Committee informed the Secretary on April 29, 1958 that Dr. Elery R. Becker, Arizona State College, Tempe, Arizona, was willing to serve and was the Committee's unanimous nominee. The Council, by mail vote on an April 30, 1958 communication from the Secretary, elected Dr. Becker to a 5-year term as Editor starting January 1, 1959.

4. *Committee on Journal Index* (*W. S. Bailey*): The Council, during its Forty-Seventh Meeting, authorized Dr. Bailey to apply to the National Science Foundation for a grant of $3410.00 to cover one-half of the estimated cost of preparing an index for Volumes 26 through 44 of the Journal, with the other one-half to be provided from funds in the office of the Custodian of Back Issues. The proposal was to prepare an index similar to the index for Volumes 1–25 which was printed in 1941. Dr. Bailey reported that the N.S.F. had approved the application and that the Treasurer has received a check for $3410.00 from the N.S.F. for this project.

The report was accepted and ordered placed on file.

5. *Committee on Russian Translations* (*K. C. Kates, Chr., G. Anastos, E. C. Dougherty, M. Doss*): Considerable impetus has developed during the year in regard to translation projects on foreign scientific literature, particularly Russian. The National Science Foundation, the A.I.B.S., and the National Institutes of Health have been active in the field of biological sciences. A committee of the United States Senate is giving serious attention to the problem.

During the 1957 ASP meeting, the Society's President was requested by the A.I.B.S. to recommend a Russian parasitological journal, as well as important monographs in the field, which should be given first priority for translation into English. The responsibility for making such recommendations was delegated to this Committee. The following recommendations were made: *Journal: Trudy Gel 'mintologicheskoi Laboratorii;* Academiia Nauk SSSR. *Monographs:* (1) *Opredelitel' paraziticheskikh nematod;* edited by K. I. Skrjabin; vol. 3, 1953; Strongiliaty (Strongylata), 890 pp. (*Note:* Part of this volume has already been privately translated, so completing the translation of this volume would cost less than translating one of the other volumes of this series from scratch); (2) *Trematody zhivotnykh i cheloveka* (Trematodes of animals and man); edited by K. I. Skrjabin; vol. 5, 1952; *Schistosomata,* 624 pp.; (3) *Anoplotsefaliaty-Lentochnye gel' minty domashnikh i dikikh zhivotnykh* (Anoplocephalata—cestodes of domestic and wild animals); A. A. Spasskii; vol. 1, 1951, 735 pp. A further recommendation was made that the A.I.B.S. consider the preparation and publication of a comprehensive, Russian-English Biological Dictionary.

These recommendations were made to the A.I.B.S. about a year ago and were well received. The A.I.B.S. is making some progress in obtaining funds with which to include some translation of parasitological literature in the A.I.B.S. program.

The A.I.B.S. believes it can perform a very valuable service to biologists generally by establishing an *A.I.B.S. Clearing House of Russian Scientific Information in Biology.* This Committee believes such a clearing house within the A.I.B.S. is a proper function of this organization, and would perform an important service to parasitologists worthy of the Society's support. How the Society can aid in the establishment of such a clearing house within the A.I.B.S. is indicated below in the Committee's recommendations.

During the year the Index Catalog of Medical and Veterinary Zoology, Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Center, Beltsville, Md., has continued to index increasing quantities of Russian parasitological literature. Supplement No. 8 of the Author Catalogue was published during the year. Plans have also been made to publish the Parasite Catalogue, Host Catalogue, Checklist of Generic and Specific Names, and the Treatment Catalogue, possibly on Microcards, and a demonstration of sample Microcards of these 5 additional catalogues will be presented at this meeting. Financing of the publication of these 5 additional catalogues has not yet been arranged, but it is hoped that this can be accomplished in the near future.

During the year Miss Judith M. Humphrey, a member of the staff of the Index Catalogue of Medical and Veterinary Zoology, working under the supervision of Miss Mildred A. Doss, has begun the preparation of several glossaries of Russian parasitological terms in which both the Russian-English and English-Russian translations will be listed. The first glossary—*Glossary of Nematological Terms*—has been completed and a copy is attached to this report. Other similar glossaries on the Trematoda, Cestoda, Acanthocephala and Protozoa will be prepared in the near future. Copies of these glossaries will be available upon request when completed from the Index Catalogue of Medical and Veterinary Zoology.

It is recommended: (1) That the American Society of Parasitologists support, by direct recommendations to the N.S.F., the establishment within the A.I.B.S. of a "*Clearing House for Russian Scientific Information in Biology*". (2) That the Council recommend to the State Department, as a part of their Cultural and Scientific Exchange Program with Russia, that it would be desirable to arrange for an American-Russian exchange group of parasitologists, which could be sponsored jointly by the State Department and the American Society of Parasitologists.

The report was accepted and ordered placed on file.

6. *Committee on Honorary and Emeritus Members* (*L. J. Olivier, Chr., G. H. Ball, A. M. Fallis, L. A. Jackowski, R. B. McGhee, C. P. Read*): The Committee, having ascertained that vacancies occur within the requirements of the Society's By-Laws, unanimously nominated Professor Rupert Montgomery Gordon, Liverpool School of Tropical Medicine, as an Honorary Member and Dr. David Henry Wenrich, University of Pennsylvania, as an Emeritus Member.

The report was accepted and ordered placed on file.

7. *Committee on A.S.P.-A.I.B.S. Relationships* (*P. E. Thompson, Chr., W. S. Bailey, L. Jacobs*): This Committee was appointed, by former President G. F. Otto subsequent to the Forty-Seventh Council Meeting, to represent the Society when the A.I.B.S. held a meeting relative to ways that it might participate in reducing the clerical and business burdens of the Society's Custodian, Editor, Secretary, and Treasurer. Since a meeting has not been called by the A.I.B.S., the Committee reported that it had not been able to take any action during the year.

The report was accepted.

V. Old Business

There were no items pertaining to old business.

VI. New Business

1. *Bonding of Custodian:* The new By-Laws require that the Custodian be bonded (the Treasurer had been bonded prior to the new instrument). The new Treasurer (Dr. M. J. Ulmer) was instructed to apply for a blanket bond of $10,000 to cover both the Custodian and Treasurer.

2. *Transfer of Endowment Fund to Treasurer.* The Secretary was instructed to request Dr. N. R. Stoll (former Custodian of the Endowment Fund) to transfer this account to the Treasurer of the Society.

3. *Solicitation of A.I.B.S. Support for Publishing Index Catalogue of Medical and Veterinary Zoological Literature:* The Secretary was instructed to write a letter in behalf of the Society requesting the above support.

4. *A Society Seal.* The Council empowered the President to appoint a Committee to submit a potential design of an official seal for the Society. It was agreed that this committee also

would work on the design of a research award medal if the Society should vote to have this.

5. *Society Affiliation by Annual Midwestern Conference of Parasitologists.* Following appropriate petition as set forth under Article VI of the By-Laws and personal representation by Dr. F. J. Kruidenier, the Council voted unanimously to accept the Midwestern Conference of Parasitologists as an Affiliate of the Society.

6. *Election of New Members.* Information on 10 applicants was submitted to the Council; all of the applicants were elected to active membership.

7. *A Research Award in Parasitology.* The Council agreed to recommend to the Society the institution of a research award in parasitology as outlined in the above Report of the Committee on Awards.

8. *A.I.B.S. Proposals to Society.* The Council voted to recommend to the Society adoption of the following A.I.B.S. proposals (see Report of Representative to A.I.B.S. Governing Board above): (1) That the A.I.B.S. Council be enlarged to include 4 Council Members at Large, (2) that member Societies be assessed annually $1.00 per capita rather than a maximum of $1000.00 as at present.

9. *Elections to the Editorial Board.* The Council elected for 4-year terms G. C. Goodchild and R. M. Cable to succeed themselves and R. E. Thorson to succeed P. E. Thompson.

10. *Appointments to Standing Committees:*

(a) *Visual Education and Instruction:* M. S. Ferguson, Chr., D. V. Moore, J. C. Swartzwelder (Committee continued with no change in personnel).

(b) *Nomenclature and Terminology:* To be named by the President.

(c) *In Memoriam:* To be named by the President.

(d) *Public Relations:* To be named by the President.

(e) *Awards:* Pending Society action on the award proposal, this committee is to be named by the President.

(f) *Tellers:* To be named by the President.

(g) *Honorary and Emeritus Members:* To be named by the President.

(h) *Resolutions:* To be named by the President.

11. *Appointments to Ad Hoc Committees.*

(a) *Career Booklet:* D. V. Moore, Chr. (Committee continued with no change in personnel except for A. C. Chandler who it was subsequently learned has passed away.)

(b) *Procurement of Personnel to Prepare Journal Index:* M. Doss, Chr., A. O. Foster, A. McIntosh.

(c) *Supervision of Preparation of Journal Index:* To be named by the President.

(d) *Auditing Committee:* (This Committee's function will be only to audit the Financial Report of the Custodian as the auditing of the other funds has been provided for as indicated above.) To be named by the President.

(e) *Russian Translations:* To be named by the President with the request to serve simply on a standby basis rather than to be charged with a specific course of action.

12. *Selection of Meeting Sites for Future Annual Meetings.* The Council made the following proposals.

1959 Meet with the A.I.B.S., Pennsylvania State University, State College, Pennsylvania, last week in August.

1960 Meet with the American Society of Tropical Medicine and Hygiene, Los Angeles, California, November 2–5. (Subject to ASTMH ratification.)

1961 and 1962 Probably with the A.I.B.S., but places and dates not yet available.

1963 Possibly with American Society of Tropical Medicine and Hygiene, Chicago, Illinois, in early November.

13. *Louisiana State University Fellowship Program in Parasitology and Tropical Medicine.* Dr. H. E. Meleney transmitted to the Council a special announcement regarding the availability of fellowships. It was agreed by the Council to bring this announcement to the attention of the Society during the annual business meeting.

14. *Miscellaneous:* In response to a request by the National Research Council that the Society solicit comments on "An Approach Toward A Course in The Principles of Parasitism" (J. Parasitol. **44**: 28, 1958), the Council instructed the Secretary to offer either to insert an appropriate statement in the Journal or to supply a list of the membership to the N.R.C. for its use in a direct questionnaire.

The Council voted that the amounts of Society funds used by officers of the Society in attending the annual meeting should appear in the Treasurer's records.

The Council voted travel allowance for Dr. M. J. Ulmer to go from Ames to Denver in connection with assuming the office of Treasurer.

The Council voted a $100.00 travel allowance to the Custodian for use in attending annual meetings, effective in 1958.

No action was taken on a proposal that the Society operate a placement bureau desk during annual meetings.

The Secretary was instructed to request that the Chairman of the Public Relations Committee attend, if possible, the International Congress on Scientific Information, Washington, D.C., November 16–21, 1958.

The Council rejected a proposal that foreign membership dues be increased over domestic membership dues to cover difference in mailing costs of the Journal.

The Council took no action on a proposal to institute a new mechanism for collecting author's charges that are incurred in publishing in the Journal.

The Secretary was instructed to write letters expressing the Society's appreciation for the services of Dr. R. M. Stabler as Treasurer, to Dr. N. R. Stoll as Custodian of the Endowment Fund, and to Dr. L. Jacobs as Editor.

The Secretary was instructed to write a letter of acceptance and appreciation to Parke, Davis and Company for the sponsorship of a research award in parasitology.

The President was requested to send a letter of appreciation to Mrs. Gerry Jacob for exceptional service in indexing and binding of the Society's archives.

The Council voted to adjourn at 11:25 P.M., August 28, 1958.

Respectfully submitted,
/s/ Paul E. Thompson, *Secretary*

AMERICAN SOCIETY OF PARASITOLOGISTS

Thirty-Third Annual General Business Meeting, August 27, 1958

The General Business Meeting of the Society was called to order by President Walton at 1:45 P.M. following the annual luncheon held in the Union Building, Indiana University.

1. The status of the membership and of the financial affairs of the Society were summarized briefly.

2. The affairs of the Custodian's office were reviewed and the plans for indexing Volumes 26–40, with the aid of a N.S.F. grant, were announced.

3. A brief report on the Journal was presented. Upon a motion from the floor, the Society expressed its appreciation to Dr. Leon Jacobs for his services as Editor.

4. The availability of a Society-managed annual award for meritorious research in parasitology, sponsored by Parke, Davis and Company, and consisting of a check for $1000.00, a medal designed by the Society, and $150.00 travel allowance for the recipient were announced. The Council recommended that the Society accept the offer of the sponsor, that the award be named in honor of Henry B. Ward, that the recipient be a member of the Society whose accomplishment occurs within 15 years after the completion of academic training, and that the recipient be selected by a presidentially appointed committee (designated the Committee on Awards) of 5 members representing the several disciplines within the Society. Members of the Committee would normally serve 2-year terms and would not be eligible for consecutive terms.

The Society voted to accept the sponsor's offer and the foregoing recommendations of the Council pertaining to the award.

5. Plans for work on an official Society Seal and for the design of the Henry B. Ward Medal were announced. Dr. Justus F. Mueller was designated as Chairman of the Seal and Medal Committee, whose function would be to receive from Society members and propose possible designs for Society acceptance.

6. As a member society of the A.I.B.S. the Society was asked to vote on proposals by the A.I.B.S. that it be empowered (1) to enlarge its council by including 4 council members at large, and (2) to increase the annual dues of member societies to $1.00 per capita rather than a maximum of $1000.00 per member society. The Society's Council recommended that the Society vote in favor of these proposals, and the Society so voted.

7. Upon recommendation by the Council, the Society elected Professor Rupert Montgomery Gordon an Honorary Member and Dr. David Henry Wenrich an Emeritus Member.

8. Dr. N. R. Stoll gave a report as a Society delegate to the 15th International Congress of Zoology and the 7th Colloquium on Nomenclature.

9. The Council announced the following appointments:

Editor, 5-yr. term—Dr. Elery R. Becker.

Assistant Editors, 3-yr. terms—Dr. R. M. Cable, Dr. C. G. Goodchild, Dr. R. E. Thorson.

10. The Nominating Committee, Dr. H. W. Stunkard, Chr., submitted the following slate of nominees:

President-Elect—Dr. H. W. Brown
Vice-President—Dr. R. M. Stabler
Treasurer, 2-year term—Dr. M. J. Ulmer
Council Members at Large, 4-yr. terms—Dr. R. V. Bangham, Dr. L. J. Olivier

An invitation was made for nominations from the floor, and there were no additional nominations. Action was then requested, and the Society voted for the slate of nominees listed above.

11. Following a call for nominations from the floor, the Society elected Dr. John S. Andrews as Chairman of the Nominating Committee.

12. President Walton announced that the Society's Council was pleased to accept the petition of the Annual Conference of Midwestern Parasitologists as an Affiliate of the Society.

13. Future meeting plans were announced as follows:

1959 with the A.I.B.S., Pennsylvania State University, State College, Pennsylvania, August 30–September 2.

1960 with the American Society of Tropical Medicine and Hygiene, Los Angeles, California, November 2–5. (Final confirmation of this joint meeting will depend upon official agreement by the Council of the American Society of Tropical Medicine and Hygiene.)

Specific plans for the 1961 and 1962 meetings were not available, but consideration was suggested for a possible joint meeting with the American Society of Tropical Medicine in 1963, in Chicago, Illinois.

14. A communication from Dr. H. E. Meleney regarding the availability of fellowships in the Louisiana State University Fellowship Program in Parasitology and Tropical Medicine was presented to the Society.

15. Dr. J. E. Ackert offered the following resolution: "Whereas, in the passing of Dr. Asa C. Chandler, the Society has lost a charter member, a past president, an active member of the Editorial Board and a productive contributor to our programs; and whereas, his passing has removed an inspiring teacher, a discerning investigator, an eminent writer and author and an able administrator;

Therefore be it resolved that the Society express its deep sense of loss in the death of Dr. Chandler;

Be it further resolved that this expression become a part of the permanent records of the Society, and that a copy of this resolution be sent to the members of his family."

The resolution was accepted with sorrow by the Society.

16. A resolution was voted expressing the Society's appreciation for the services of Dr. Leon Jacobs as Editor, Dr. R. M. Stabler as Treasurer, and Dr. N. R. Stoll as Custodian of the Endowment Fund. A resolution of appreciation also was voted for the contributions in connection with the annual meeting of the A.I.B.S., Indiana University, and Local Committee Representatives Dr. Shelby D. Gerking and Mr. M. C. McCowen.

17. The Society voted to adjourn at 2:25 P.M.

Respectfully submitted,
/s/ Paul E. Thompson, *Secretary*

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